University of Alberta

Extraction and Functional Properties of Barley Proteins

by

Arun Kumar Lekhi ©

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Master of Science

in

Food Science and Technology

Department of Agricultural, Food and Nutritional Science

Edmonton, Alberta

Spring, 2004

Library and
Archives Canada

Published Heritage
Branch

395 Wellington Street
Ottawa ON K1A 0N4
Canada

Bibliothèque et
Archives Canada

Direction du
Patrimoine de l'édition

395, rue Wellington
Ottawa ON K1A 0N4
Canada

Your file Votre référence
ISBN: 978-0-494-22421-2
Our file Notre référence
ISBN: 978-0-494-22421-2

NOTICE:
The author has granted a non-exclusive license allowing Library and Archives Canada to reproduce, publish, archive, preserve, conserve, communicate to the public by telecommunication or on the Internet, loan, distribute and sell theses worldwide, for commercial or non-commercial purposes, in microform, paper, electronic and/or any other formats.

The author retains copyright ownership and moral rights in this thesis. Neither the thesis nor substantial extracts from it may be printed or otherwise reproduced without the author's permission.

AVIS:
L'auteur a accordé une licence non exclusive permettant à la Bibliothèque et Archives Canada de reproduire, publier, archiver, sauvegarder, conserver, transmettre au public par télécommunication ou par l'Internet, prêter, distribuer et vendre des thèses partout dans le monde, à des fins commerciales ou autres, sur support microforme, papier, électronique et/ou autres formats.

L'auteur conserve la propriété du droit d'auteur et des droits moraux qui protège cette thèse. Ni la thèse ni des extraits substantiels de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation.

In compliance with the Canadian Privacy Act some supporting forms may have been removed from this thesis.

While these forms may be included in the document page count, their removal does not represent any loss of content from the thesis.

Conformément à la loi canadienne sur la protection de la vie privée, quelques formulaires secondaires ont été enlevés de cette thèse.

Bien que ces formulaires aient inclus dans la pagination, il n'y aura aucun contenu manquant.

Canada

University of Alberta

Library Release Form

Name of Author: *Arun Kumar Lekhi*

Title of Thesis: *Extraction and Functional Properties of Barley Proteins*

Degree: *Master of Science*

Year this Degree Granted: *2004*

Permission is hereby granted to the University of Alberta Library to reproduce single copies of this thesis and to lend or sell such copies for private, scholarly or scientific research purposes only.

The author reserves all other publication and other rights in association with the copyright in the thesis, and except as herein before provided, neither the thesis nor any substantial portion thereof may be printed or otherwise reproduced in any material form whatever without the author's prior written permission.

1051 James Crescent
Edmonton, Alberta
Canada
T6L 6P6

Date: January 30, 2004

University of Alberta

Faculty of Graduate Studies and Research

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled **Extraction and Functional Properties of Barley Proteins** submitted by **Arun Kumar Lekhi** in partial fulfillment of the requirements for the degree of **Master of Science** in **Food Science and Technology**.

Dr. Feral Temelli (Supervisor)

Dr. Thava Vasanthan (Committee member)

Dr. Jim Unterschultz (Committee member)

Date: Jan. 30/2004

ABSTRACT

Barley proteins may possess unique properties that could be utilized in value-added products. Proteins were extracted from Candle (waxy) and Condor (regular) barley grains, quantified and their functional properties were determined. Starch and β-glucan were concentrated in the pearled grain flour (19% PGF) while protein, lipid and ash were concentrated in the pearling flour (19% PF) with the proteins having a favorable amino acid profile. The NaOH extraction at pH 11/23°C resulted in the highest ($P \leq 0.05$) protein recovery and purity whereas β-glucan and starch could be isolated with NaOH or Na_2CO_3 at pH 11/50°C. The hordein content (42-45%) was highest in 19% PGF while 19% PF of Condor contained 64% of globulins. Barley protein concentrates have high hydration capacity (4-7 g water/g protein), foaming capacity (200-437%) and stability (72-88%) and can form stable emulsions. Alkali-extracted barley proteins show potential as functional and nutritional ingredients in various foods products.

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to my supervisor, Dr. Feral Temelli for all her support and guidance. Her positive attitude has been a tremendous motivational factor in helping me complete my thesis. A personal thanks to Dr. Thava Vasanthan for his insights and suggestions provided throughout my studies. Thanks to Dr. Jim Unterschultz for taking time out of his busy schedule to be on my defense committee.

I would like to thank the Alberta Barley Commission and the Alberta Agricultural Research Institute for financial support of this project. My appreciation goes to Agricore United and Dr. Jim Helm for providing me with the Candle and Condor barley, respectively. Thanks also to Dr. Kevin Swallow and the rest of the staff at the Leduc Food Processing Development Centre for all their assistance during pilot scale processing of the proteins.

I would like to extend my appreciation to Li Sun for all her valuable technical support and assistance in helping me complete this project. I would like to thank Dr. Karamat Rezaei, Judy Yeung and Paul Moquin for their constant friendship and providing me with helpful ideas throughout the study. Thanks is also given to Len Steele and Jean Bourgois for training me on the various instruments in the lab.

Thanks to all the professors, staff and colleagues at the Department of Agricultural, Food and Nutritional Science. Finally, thanks to my Tiajee, Sat Pal Lekhi and the rest of my family for always encouraging me to achieve my academic goals.

To my beloved wife, Mamta, my son, Nathan, daughter, Serena, niece, Alicia, brother, Trun and my parents (Jai and Prem). Their love, understanding and compassion has given me the strength to keep reaching for new heights.

TABLE OF CONTENTS

Title **Page**

1. INTRODUCTION AND THESIS OBJECTIVES 1

2. LITERATURE REVIEW 4
- 2.1. BARLEY 4
  - 2.1.1 Production 5
  - 2.1.2. Utilization 5
  - 2.1.3. Seed structure and composition 7
  - 2.1.4. Health benefits of barley 9
- 2.2. BARLEY PROTEINS 11
  - 2.2.1. Classification of protein types 12
  - 2.2.2. Structure and composition 12
  - 2.2.3. Nutritive value of barley proteins 15
  - 2.2.4. Protein extraction 19
    - 2.2.4.1. Protein separation according to Osborne solubility classes 19
    - 2.2.4.2. Alkaline extraction 22
  - 2.2.5. Functional properties 22
- 2.3. OTHER SOURCES OF PROTEIN INGREDIENTS 26
  - 2.3.1. Wheat protein structure and function 28
  - 2.3.2. Soybean protein structure and function 29
- 2.4. BARLEY β-GLUCAN 34
  - 2.4.1. Structure and function 34
  - 2.4.2. Health benefits of barley β-glucan 34
- 2.5. BARLEY STARCH 36
  - 2.5.1. Structure and function 37
  - 2.5.2. Barley flour and starch end uses 38
- 2.6. REFERENCES 39

3. QUANTIFICATION AND DISTRIBUTION OF PROTEINS IN BARLEY PEARLING FRACTIONS 48
- 3.1. INTRODUCTION 48
- 3.2. MATERIALS AND METHODS 53
  - 3.2.1. Materials 53
  - 3.2.2. Pearling 53
  - 3.2.3. Compositional analysis 54
  - 3.2.4. Scanning electron microscopy 54
  - 3.2.5. Color determination 54
  - 3.2.6. Protein fractionation 55
  - 3.2.7. Amino acid analysis 57
  - 3.2.8. Statistical Analysis 58
- 3.3. RESULTS AND DISCUSSION 58
  - 3.3.1. Compositional analysis of barley pearling fractions 58
  - 3.3.2. Scanning electron microscopy 62

3.3.3. Color analysis 62
3.3.4. Quantification and distribution of protein classes 68
3.3.5. Amino acid analysis........ 69
3.4. CONCLUSIONS 75
3.5. REFERENCES 76

4. EFFECT OF ALKALINE EXTRACTION ON THE RECOVERY AND PURITY OF PROTEINS, β-GLUCAN AND STARCH IN HULL-LESS BARLEY WHOLE GRAIN AND PEARLING FLOURS........ 80
4.1. INTRODUCTION 80
4.2. MATERIALS AND METHODS 83
4.2.1. Materials 83
4.2.2. Extraction conditions 83
4.2.3. Compositional analysis 85
4.2.4. Amino acid analysis 86
4.2.5. Statistical analysis 86
4.3. RESULTS AND DISCUSSION 86
4.3.1. Compositional analysis of barley pearling fractions 86
4.3.2. Extraction conditions 87
4.3.2.1. Protein recovery and purity 87
4.3.2.2. β-Glucan purity and recovery 92
4.3.2.3. Starch purity and recovery 94
4.3.3. Amino acid analysis 94
4.4. CONCLUSIONS 97
4.5. REFERENCES 98

5. FUNCTIONAL PROPERTIES OF ALKALI-EXTRACTED BARLEY PROTEINS 100
5.1. INTRODUCTION 100
5.2. MATERIALS AND METHODS 103
5.2.1. Materials 103
5.2,2. Functional properties 103
5.2.2.1. Differential scanning calorimetry 103
5.2.2.2. Hydration capacity 103
5.2.2.3. Nitrogen solubility 104
5.2.2.4. Emulsion stability 104
5.2.2.5. Foaming capacity and stability........ 105
5.2.3. Statistical analysis 106
5.3. RESULTS AND DISCUSSION 106
5.3.1. Proximate analysis 106
5.3.2. Functional properties 109
5.3.2.1. Differential scanning calorimetry 109
5.3.2.2. Hydration capacity 115
5.3.2.3. Nitrogen solubility 116
5.3.2.4. Emulsion stability 117
5.3.2.5. Foaming capacity and stability 120

5.4. CONCLUSIONS 123
5.5. REFERENCES 123

6. **CONCLUSIONS AND RECOMMENDATIONS** 127
6.1. REFERENCES 133

LIST OF TABLES

Table **Page**

2.1. Production of field crops in Canada 6

2.2. Field crop production in western Canadian provinces for the year 2001 6

2.3. Overall chemical composition of barley grain 10

2.4. Classification scheme to identify cereal proteins 13

2.5. Classification of barley proteins 13

2.6. Amino acid composition (g/16 g N) of regular barley proteins 16

2.7. Nitrogen digestibility values of some proteins 17

2.8. Protein quality of cereal proteins 18

2.9. Basic functional properties performed by proteins in food systems 24

2.10. World production of some major protein-based food sources and daily protein consumption 27

2.11. Proximate composition (% w/w) of soybeans, flour and protein fractions 31

3.1. Proximate analysis of Candle barley pearling fractions 60

3.2. Proximate analysis of Condor barley pearling fractions 61

3.3. Effect of pearling on the Hunter color values (*L*, *a*, *b*) of Candle and Condor pearling fractions 67

3.4. Quantification of the four major protein classes in whole grain and 19% pearling fractions of Candle and Condor barley 69

3.5. Distribution of the four major protein classes in 19% pearling fractions of Candle and Condor barley 69

3.6. Amino acid composition of whole grain flour and 19% pearling fractions of Candle and Condor barley 70

3.7. Amino acid composition of albumins (Alb), globulins (Glob), hordeins (Hord) and glutelins (Glut) in whole grain flour (WGF) of Candle and Condor barley ... 71

3.8. Amino acid composition of albumins (Alb), globulins (Glob), hordeins (Hord) and glutelins (Glut) in 19% pearling flour (PF) of Candle and Condor barley 72

3.9. Amino acid composition of albumins (Alb), globulins (Glob), hordeins (Hord) and glutelins (Glut) in 19% pearled grain flour (PGF) of Candle and Condor barley 73

3.10. Distribution of lysine in protein fractions of Candle and Condor WGF, 19% PF and 19% PGF 73

3.11. Essential amino acid content and chemical scores of whole egg, whole-wheat grain, soy protein isolates and Candle and Condor WGF, 19% PF and 19% PGF 75

4.1. Proximate analysis of Candle and Condor barley whole grain and 19% pearling flour fractions obtained from large batch of pilot processing 87

4.2. Amount of protein extracted, purity and recovery of proteins using 0.035*M* NaOH and Na_2CO_3 at pH 10 and 11 at 23°C for Candle whole grain and pearling flour fractions 88

4.3. Amount of protein extracted, purity and recovery of proteins using 0.035*M* NaOH and Na_2CO_3 at pH 10 and 11 at 23°C for Condor whole grain and pearling flour fractions 89

4.4. Amount of protein extracted, purity and recovery of proteins using 0.035*M* NaOH and Na_2CO_3 at pH 11 and at 23, 40 and 50°C for Candle and Condor 19% PGF 90

4.5. Amount of β-glucan extracted, purity and recovery of β-glucan using 0.035*M* NaOH and Na_2CO_3 at pH 10 and 11 and at 23, 40 and 50°C for Candle and Condor 19% PGF 93

4.6. Amount of starch extracted, purity and recovery of starch using 0.035*M* NaOH and Na_2CO_3 at pH 10 and 11 and at 23, 40 and 50°C for Candle and Condor 19% PGF 95

4.7. Amino acid composition of proteins extracted (0.035*M* NaOH, pH 11 and 23°C) from whole grain flour and 19% pearling fractions of Candle and Condor barley 96

5.1. Proximate analysis of Candle and Condor barley protein concentrates extracted from WGF, 19% PF and 19% PGF using 0.035*M* NaOH at pH 11 and 23°C 107

5.2. Proximate analysis of Candle and Condor barley protein concentrates extracted from 19% PGF using 0.035*M* NaOH at pH 11 and 40 and 50°C 107

5.3. Hydration capacity (g H_2O/g protein) and nitrogen solubility of protein concentrates extracted from Candle and Condor barley WGF, 19% PF and 19% PGF using 0.035*M* NaOH at pH 11 and 23, 40 and 50°C 116

5.4. Emulsion stability of protein concentrates extracted from Candle and Condor barley WGF, 19% PF and 19% PGF using 0.035*M* NaOH at pH 11 and 23, 40 and 50°C 118

5.5. Foaming capacity and foaming stability of protein concentrates extracted from Candle and Condor barley WGF, 19% PF and 19% PGF using 0.035*M* NaOH at pH 11 and 23, 40 and 50°C 121

LIST OF FIGURES

Figure **Page**

2.1. Barley grain structure. Adapted from Newman and Newman (1991), with permission from Cereal Foods World 8

3.1. Extraction of protein classes from whole grain barley flour and 19% pearling fractions 56

3.2. Scanning electron micrographs of Candle barley whole grain (A), 18% pearling (B), 22% pearling (C), and 39% pearling (D) fractions. Images are 700x magnification 63

3.3. Scanning electron micrographs of Condor barley whole grain (A), 18% pearling (B), 21% pearling (C), and 42% pearling (D) fractions. Images are 700x magnification 65

4.1. Flow diagram for the extraction of starch, protein and β-glucan from whole grain and pearling flours of Candle and Condor barley 84

5.1. DSC results for Candle barley WGF and pearling fractions 110

5.2. DSC results for Condor barley WGF and pearling fractions 111

5.3. DSC results for Candle barley protein concentrates obtained from WGF and pearling fractions at various temperatures 113

5.4. DSC results for Condor barley protein concentrates obtained from WGF and pearling fractions at various temperatures 114

1. INTRODUCTION AND THESIS OBJECTIVES

Even before the discovery of wheat, barley was consumed extensively throughout the world because of its ability to grow in various climatic conditions and it provided an important source of dispensable energy. The increased consumption of wheat over the years has been largely due to the structural behavior of its endosperm proteins. Once the starch is washed away, these proteins form an elastic and cohesive "gluten mass," which has had far-reaching implications in the baking industry. Thus, due to the widespread cultivation of different varieties of wheat and the domestication of animals, barley has been relegated to livestock feed and it has also found tremendous use in the manufacturing of alcoholic beverages.

In general, cereal grain proteins are considered to be non-nutritive due to their deficiency in essential amino acids, which mainly include lysine, methionine and threonine. When compared to wheat, barley proteins contain a higher percentage of all ten essential amino acids that are mostly concentrated in the bran, whereas the structural (storage) proteins are localized in the endosperm. Unlike wheat, barley storage proteins lack the ability to form a gluten network. However, barley proteins may exhibit other important functional and/or structural properties that may enhance their use in various food systems, but this aspect needs to be explored since the literature lacks such information.

Research data on the isolation and functional properties of barley proteins are limited. Most of the studies conducted on barley have focused on the extraction of β-glucan and starch. β-Glucan is a soluble fiber component and shows tremendous potential in reducing plasma cholesterol levels in the human body as well as regulating blood glucose levels. These health benefits of β-glucan may have positive implications for heart disease and diabetes.

Barley starch contains varying levels of amylose and amylopectin depending on the variety, which affects its swelling, gelatinization and retrogradation properties. Thus, there is great interest in determining the physico-chemical properties of barley starch to assess its compatibility (on its own or in conjunction with other cereal starches) for its utilization in starchy foods (i.e. bread, pasta and noodles) or industrial products such as paper and cardboard.

Barley proteins are underutilized as a human food ingredient. In both β-glucan and starch processing, proteins are considered as contaminants and are precipitated out and discarded. Majority of the barley proteins are distributed in two major grain tissues, the aleurone layer (bran) and the starchy endosperm. These tissues have different protein concentrations with different amino acid profiles and therefore, it stands to reason that those proteins of different tissues may also exhibit unique functional and nutritive properties that may find applications in different food products. There is limited information available on the methods to extract barley protein concentrates from various tissues of the seed and assess their functionality.

Considering the fact that Alberta and Canada are major barley producers in the world, it would be economically advantageous for barley growers if the versatility of barley can be enhanced by fractionating the grain into its major components (β-glucan, lipids, starch, and proteins) and finding high value end uses for them. Therefore, the overall objectives of this thesis were to fractionate the barley grain into protein and its other major components (β-glucan and starch) by dry methods followed by extraction and purification using wet separation techniques and to determine the functional and physical properties of the protein fraction with the goal of enhancing value-added processing of barley. The specific objectives of this thesis were:

1. to determine the optimum pearling percentage to separate protein, β-glucan and starch, to quantify the four major classes of proteins in the pearling fractions and to determine their distribution and amino acid content in whole grain, pearling flour and pearled grain flour using waxy and regular barley varieties, Candle and Condor, respectively (Chapter 3),
2. to determine the effects of solvent type, pH and temperature on the extraction yield and purity of proteins, β-glucan and starch in whole grain, pearling flour and pearled grain flour of both varieties (Chapter 4), and
3. to determine the functional properties of the protein-enriched fraction isolated from whole grain, pearling flour and pearled grain flour of both varieties obtained under the best extraction conditions (Chapter 5).

2. LITERATURE REVIEW

2.1. BARLEY

Barley (*Hordeum vulgare*) is known as the "ancient crop". Records indicate that barley was cultivated in Egypt between 5,000 and 6,000 B.C (Pomeranz 1974). Barley is considered to be a versatile cereal because of its ability to grow in a wide range of environmental and soil conditions. Thus, in the early 1900's, spring barley and winter rye were heavily cultivated in most European countries. In Denmark, barley was one of the main food sources and diets were comprised of porridge of barley grits in milk or beer, and pearled barley grain in numerous meat broths (Munck 1981). Ultimately, when sustainable yields of winter and spring wheat were achieved, the consumption of barley as a human food source decreased substantially since the 1940s. Due to the functional properties of wheat starch and proteins, especially in baked goods, wheat, along with rice and corn became the food cereals of choice around the world. As nations progressed, the utilization of grain in bread altered from barley to rye and then to wheat (Newman and Newman 1991).

Even though the consumption of barley as a food source has declined over the years, it is still grown in amounts comparable to wheat. Eventually, the domestication of animals has resulted in the increased use of hull-less barley as livestock feed, whereas, hulled varieties have become popular grains in the malting, brewing and distilling industries. In recent years, the popularity of barley has been increasing due to the nutritional and functional value of its dietary fiber for human health. Thus, the utilization of barley in the human diet (through various food applications) is expected to rise in the near future.

2.1.1. Production

Among the cereal grains, barley accounts for 12% of the world's cereal production, which is ranked fourth after wheat, rice and corn (Jadhav et al 1998). Following wheat, the second highest crop produced in Canada is barley and Canada is one of the largest producers and exporters of barley in the world. An estimated output of 12 million tonnes of barley is expected for the 2003 crop year (Atkinson 2003) which is a 7% increase as compared to the year 2001 (Table 2.1). In the province of Alberta, similar amounts of wheat and barley are grown (6 and 5 million tonnes, respectively), with Alberta contributing almost 50% (Table 2.2) of Canada's total barley production. The high degree of barley cultivation in Alberta is due to its fertile soil, clean environment, and good climate (Eliason 1996).

Western Canada (Alberta, Saskatchewan, and Manitoba) produces approximately 90% of Canada's total barley and over 50 barley varieties are registered and grown for the purposes of malting, animal feed and general use (Eliason 1996). Many different types of barley can be produced, which may be hulled or hull-less, two- or six-rowed, normal- or high-lysine, regular- or high-amylose and low- or high-β-glucan content (Jadhav et al 1998). Due to their uniform kernel size, shape and plumpness, two-rowed barley varieties account for 50% of the total land seeded with barley in Alberta (McLelland 2002).

2.1.2. Utilization

Currently, in Canada, 75% of barley is used for feed (swine, cattle and poultry), 25% for malting and less than 5% is used in food products (Bhatty 1986). Hull-less barley

Table 2.1. Production of field crops in Canada[1].

Field Crop	Production (thousand tonnes)				
	1997	1998	1999	2000	2001
Wheat	24,280	24,082	26,940	26,804	21,282
Barley	13,527	12,708	13,196	13,468	11,354
Oats	3,484	3,957	3,641	3,389	2,796
Rye	320	408	386	260	194
Corn	7,179	8,952	9,161	6,286	8,170

[1] Adapted from Statistics Canada (2001).

Table 2.2. Field crop production in western Canadian provinces for the year 2001[1].

Province	Production (thousand tonnes)			
	Wheat	Barley	Oats	Rye
Alberta	6,110	5,225	592	34
Saskatchewan	10,188	3,697	1,033	55
Manitoba	3,518	1,284	771	41

[1] Adapted from Statistics Canada (2001).

is mainly used for animal feed because it has a high metabolisable energy (for growth and development), which is obtained from sugars and starch. In barley, lysine is a limiting amino acid, but with the development of high-protein and high-lysine varieties, feed supplementation with other protein sources (i.e. soybean) would not be needed (Munck 1981).

About 25% of barley production is devoted to the manufacturing of malt for use in food and mainly for beer production (Bamforth and Barclay 1993). Specific varieties of hulled barley within the two-rowed and six-rowed types are used for the production of malt. In the food industry, malt syrup is mainly used for its color, flavor and enzyme activity. Use of malt syrup can be found in products such as breads, milk (i.e. Ovaltine), pancake/waffle batter and malt vinegar (Matz 1991).

The characteristic properties desired in beer such as color, foam, and flavor make barley an ideal grain over other cereals, such as sorghum and wheat (Bamforth and Barclay 1993). Hulled barley is used in the beer making process because the hulls protect the kernel from physical damage during the malting process and serve as a filtering aid during the separation of wort (Pomeranz 1973). Thus, by manipulating the starch, β-glucan and protein contents of barley, numerous types of malt (beer) and distilled spirits are possible to satisfy various human tastes (Munck 1981).

In the Canadian diet, wheat represents 90% of all the grains consumed, with rice, corn and oats representing between 3 to 7% (Conrad 1999). The potential for utilization in a wide variety of food products exists for barley, yet, only 5% of the barley produced is used for food applications. Barley products include muffins, cookies, pasta, noodles, biscuits and breakfast cereals (Newman and Newman 1991; Berglund et al 1992). Dietary fibre has received a lot of scientific attention and has been linked to cholesterol reduction, cancer prevention and maintaining the overall health of the gastrointestinal tract. Barley is high in soluble dietary fibre (mainly β-glucan), enhancing its nutritional value, thus making it a desirable grain (Newman and Newman 1991) for human consumption.

2.1.3. Seed structure and composition

Barley grain or kernel is called a *caryopsis*, which is a dry, one seeded fruit (Hoseney, 1994). In hulled barley, during early stages of grain development, the pericarp produces a cementing material that adheres the palea and lemma (flowering glumes) to the grain, thus forming the husk (hull) (Duffus and Cochrane 1993). The hull can be removed during processing, or alternatively, hull-less varieties are also available. Figure 2.1 shows the structure of a typical barley grain. The pericarp, seed coat and aleurone (including the

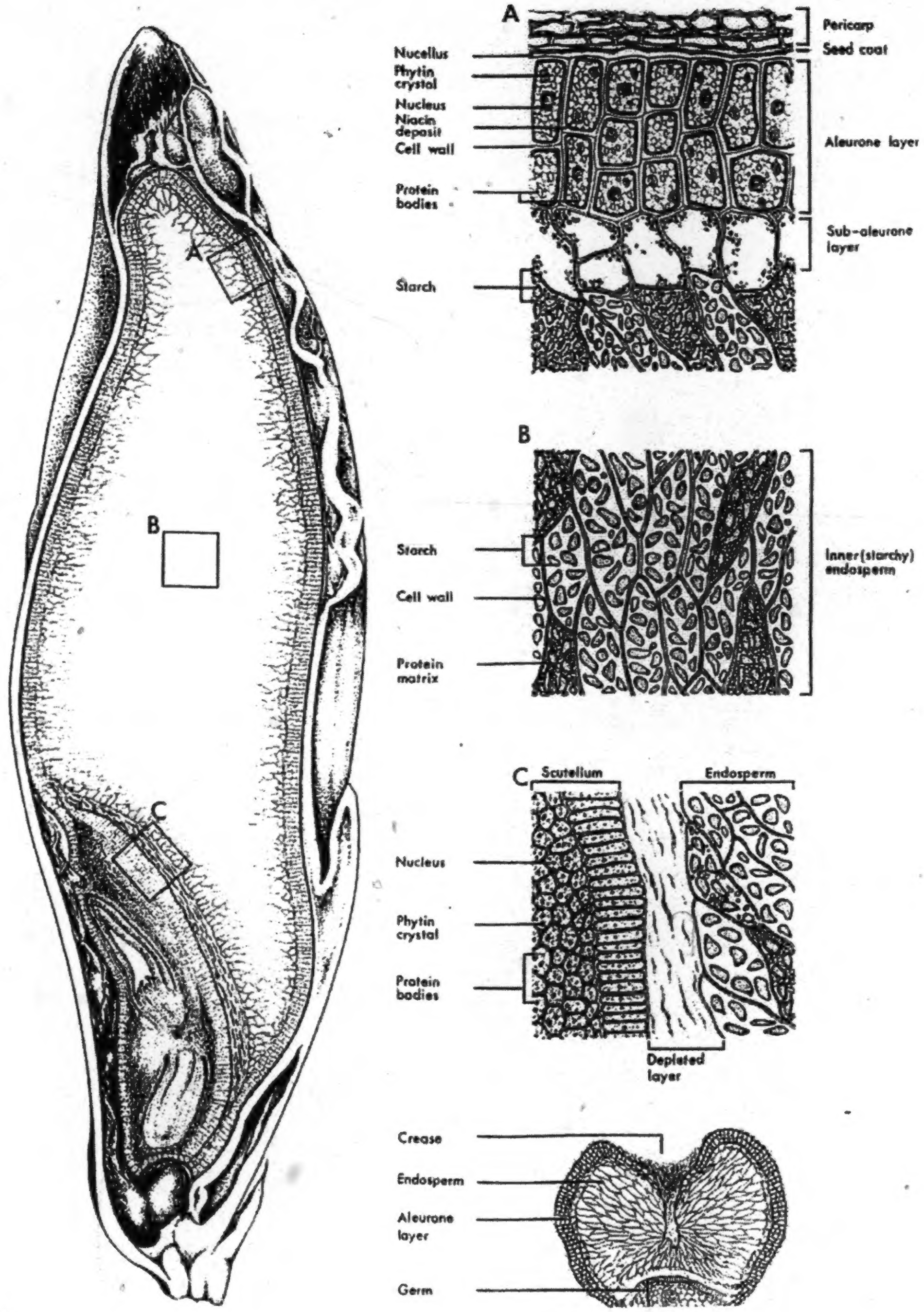
A
B
C
A
Nucellus
Phytin crystal
Nucleus
Niacin deposit
Cell wall
Protein bodies
Starch
Pericarp
Seed coat
Aleurone layer
Sub-aleurone layer
B
Starch
Cell wall
Protein matrix
Inner(starchy) endosperm
C
Scutellum
Endosperm
Nucleus
Phytin crystal
Protein bodies
Depleted layer
Crease
Endosperm
Aleurone layer
Germ

Figure 2.1. Barley grain structure. Adapted from Newman and Newman (1991), with permission from Cereal Foods World.

sub-aleurone layer) form the bran. In barley, the aleurone and sub-aleurone layers represent 8-13% (w/w) of the grain and combined, they are three to four cells thick. The bran contains approximately 20% oil, 20% minerals, 20% protein and 10% sugars (Kent 1975). Also, when present, anthocyanin pigments located in the bran are responsible for the color of the grain. Anthocyanins are red in the pericarp and blue in the aleurone layer (Pomeranz 1987).

The endosperm contains 75-85% starch, which is embedded in a protein matrix. The cell walls of the endosperm contain mixed linkage β-glucan, arabinoxylan (pentosans) and small amounts of protein. The embryo (germ) of barley is high in protein, oil, B-complex vitamins (thiamin, pyridoxine, riboflavin and pantothenic acid) and vitamin E (present as a mixture of tocopherols and tocotrienols solubilized in the oil) (Matez 1991; Newman and Newman 1991). Table 2.3 highlights the major chemical components of a typical barley grain. It should be noted that the grain composition is highly dependent on genetics and environmental conditions. Through gene manipulation and controlling certain aspects of soil chemistry, a desired end product can be obtained for a specific use.

2.1.4. Health benefits of barley

The functional food and nutraceutical industry is growing at a very fast rate in Canada, USA, Europe and Japan. Health Canada (1997) defines functional foods as "actual food products that have certain health benefits. They are similar in appearance to conventional foods, are consumed as part of a usual diet, and have demonstrated physiological benefits and/or reduces the risk of chronic disease beyond basic nutrition functions". Therefore, as research on barley continues, fractions such as β-glucan and

Table 2.3. Overall chemical composition of barley grain[1].

Component	**% (w/w) (dry wt. basis)**
Carbohydrates	78-83
Starch	63-65
Sucrose	1-2
Other sugars	1
Water-soluble polysaccharides	1-1.5
Alkali-soluble polysaccharides	8-10
Cellulose	4-5
Lipids	2-3
Protein	8-13
Albumins and globulins	2.5-4
Prolamins (Hordeins)	2.5-4
Glutelins	3-5
Non protein nitrogen (nucleic acids)	0.2-0.3
Minerals	2
Others	5-6

[1] Adapted from Pomeranz (1987) and MacGregor and Fincher (1993).

barley oil (obtained from bran and germ) have the potential of being categorized as functional ingredients for various food applications. Starch and β-glucan will be discussed further in sections 2.4 and 2.5.

There is a growing public demand for functional foods to improve health and well being. It is quite evident that consumer education will lead to increased demand for certain food products, which meet these specific criteria (Chaudhari 1997). According to a 1997 survey conducted by the Food Marketing Institute, 70% of consumers state that their diets can be more healthy and that they would rather seek alternative therapies over increased visits to their family doctors (Health Canada 1997). In recent years, a tremendous amount of research has been carried out in trying to isolate and identify the various components in barley to determine if they impart any human health benefits or enhance the functionality of other food systems. Dry milling, wet extraction and combination of both procedures are used to obtain various barley fractions, which include the bran, pearled grain flour, starch, β-glucan, protein and oil.

Specific components in barley are responsible for its nutritive value. The major component, starch, is a source of highly digestible energy. The proteins of barley have a favorable amino acid profile and contains lysine (limiting amino acid in cereal grains) at levels higher than that in corn and wheat (Matz 1991). Tocopherols and tocotrienols, which are present in amounts of 59-62 mg/kg of grain, have been reported to behave as antioxidants and suppress excess cholesterol production in the liver of chickens, swine and human test subjects (Qureshi et al 1986). It has been postulated that d-α-tocotrienol binds to and inhibits the function of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, which is a rate-limiting enzyme for cholesterol biosynthesis (Qureshi et al 1986). Also, barley β-glucan has hypocholesterolemic properties (Newman and Newman 1991) and its viscous nature in water is a very important parameter for the regulation of blood glucose levels in diabetics.

2.2. BARLEY PROTEINS

Barley proteins can be found in the aleurone layer, endosperm and the germ tissues. On average, the protein content of barley is in the range of 8 to 16% (calculated as %N x 6.25). The protein content in barley is dependent on genetic variation, cultivar type (i.e. hull-less/hulled and two/six-rowed) and environmental growing conditions, which also include soil chemistry. Kirkman et al (1982) conducted experiments where increasing nitrogen fertilization from 50 kg/ha to 200 kg/ha resulted in a 64% increase of barley proteins in the seed. The class of proteins, their structure, composition and quantity will greatly affect the nutritive quality and functional characteristics of the grain.

2.2.1. Classification of protein types

Table 2.4 highlights the classification scheme used to identify cereal proteins. Based upon solubility, barley proteins can be divided into four major groups. The albumins make up the water-soluble portion, the globulins are salt soluble whereas the hordeins (prolamins) and glutelins are soluble in alcohol and alkali, respectively. Cereal proteins, including those of barley, are classified as simple proteins because they yield only amino acids upon hydrolysis (Table 2.5). Other types include conjugated and derived proteins. Conjugated proteins (complex proteins) are those in which non-protein compounds (i.e. lipids, nucleic acids, carbohydrates and phosphates) are bound to the protein. Derived proteins are those obtained by chemical or enzymatic methods and are separated into primary (i.e. coagulated casein in the cheese making process) and secondary (i.e. peptides and peptones) derivatives (de Man 1999). Thus, barley protein fractions obtained by wet chemical extraction would be considered derived and simple proteins.

2.2.2. Structure and composition

The primary structure and compositional properties of a particular protein is directly dependent on the chemical nature of the side chain (R-group) and how the amino acids are linked together through peptide bonding. The side chain is characterized by group type (i.e. hydroxyl, amides, sulfur, hydrocarbons, aromatic and cyclic), acidity (i.e. acidic, basic and neutral) and polarity (polar and non-polar). The spatial arrangement of polypeptide chains results in the formation of primary (linear sequence of amino acids), secondary (folding of certain segments of primary structure), tertiary (folding of secondary

Table 2.4. Classification scheme[1] to identify cereal proteins.

On the basis of morphology	On the basis of biological function	On the basis of solubility	On the basis of chemical composition
Proteins of endosperm	Metabolically active (cytoplasmic proteins)	Albumins	Simple proteins
Proteins of aleurone layer	Enzymes	Globulins	Complex proteins
Proteins of the embryo	Membrane proteins	Prolamins	Lipoproteins
	Ribosomal proteins	Glutelins	Glycoproteins
	Regulatory proteins		Nucleoproteins
	Storage proteins Low molecular weight High molecular weight		

[1] Adapted from Lasztity (1984).

Table 2.5. Classification of barley proteins.

Solubility	Type of protein	Morphology	Biological function	Chemical composition
Water	Albumin (Leucosins)[1]	Aleurone, germ	Metabolically active (enzymes)	Simple
Salt	Globulin (Edestins)	Aleurone, germ	Metabolically active (enzymes)	Simple
Alcohol	Prolamin (Hordeins)	Endosperm	Storage (low molecular weight)	Simple
Alkali	Glutelin	Endosperm	Storage (high molecular weight)	Simple

[1] Terms in parenthesis refer to specific protein names (Godon and Willm 1994).

structure into compact molecules) and quaternary (association of secondary or tertiary proteins into oligomeric units) structures (McMurry 1996). Thus, by understanding the physical nature of proteins, they can be manipulated to obtain specific functional properties. For example, under appropriate conditions, milk proteins can be curdled to manufacture cheese, soy proteins are coagulated to make tofu, and by whipping egg white proteins (albumins), foam-based foods can be prepared (Kinsella 1981).

Majority of the barley proteins are made up of hordeins and glutelins, which are concentrated mainly in the endosperm and individually they each represent 35-45% of the total proteins. On the other hand, albumins and globulins are mostly present in the aleurone (bran) layer and germ and contribute 10% and 10-20%, respectively, to the total protein content. The limiting amino acids in barley proteins (as in other cereals) are lysine and methionine. Overall, the lysine concentration is higher in the bran fraction as compared to the endosperm (Pomeranz 1973; Lasztity 1984; Linko et al 1989).

During the early development of barley grain, cytoplasmic proteins are synthesized first (in the form of protein bodies) and allocated to the aleurone. But once the need for cytoplasmic proteins diminishes, the excess nitrogen is then converted to making storage proteins in the endosperm (Duffus and Cochrane 1993). Storage proteins are also in the form of membrane-bound spherical organelles, but as the endosperm matures, the starch granules cause the compression of the protein bodies into a smooth continuous matrix, as is the case with wheat (Lasztity 1984). In other cereals, such as barley, rice, maize, millet and sorghum, the protein bodies of the endosperm only partly fuse and individual protein bodies still exist (Lasztity 1984). Wolf et al (1967) determined that the glutelins form the protein matrix, in which starch granules are embedded, and that prolamins remain as protein bodies.

Cytoplasmic (albumin and globulin) and storage (hordein and glutelin) proteins differ in structure as well as physical and chemical properties. Cytoplasmic proteins (CP) are soluble in water and salt solutions, have low molecular weight, are globular in form and the amino acid profile is more nutritive as compared to storage proteins (SP). In general, the amino acid profile of CP contains higher concentrations of lysine and arginine and lower amounts of glutamic acid and proline. In contrast, SP are soluble in alcohol and alkaline solutions, and contain both low (one polypeptide chain with intramolecular disulfide bonds) and high (many polypeptide chains interconnected by intermolecular disulfide bonds) molecular weight proteins. Table 2.6 highlights the amino acid profile of regular barley proteins and shows that SP (hordein and glutelin) are high in glutamic acid and proline but low in lysine, arginine, threonine, and tryptophan (Lasztity 1984). Also, in terms of biological function, CP of the aleurone have both a secretory and synthetic function, whereas the SP of the endosperm only have a storage function. Thus, due to the complex nature of cereal proteins, they substantially affect both the nutritive and technological values of different cereal grains.

2.2.3. Nutritive value of barley proteins

Agriculture has been the backbone of human civilization. Cereals alone have the potential of supporting the world human population in terms of calories needed. In Asia and Africa, two-thirds of the diet is based upon cereal products. Aside from containing important minerals and vitamins, cereals also provide an excellent source of inexpensive energy in the form of carbohydrates and oils (Mac Key 1981). Cereals also provide sufficient amounts of proteins, but they are of a low biological value (Pomeranz 1987).

In the human body, proteins provide an exogenous source of amino acids, which serve as building blocks for all the nitrogen containing tissues and are responsible for the biosynthesis of catalysts, such as enzymes, used in various metabolic pathways (Vanicek and Turek 1985). The nutritional quality of proteins is determined by the following four main factors: (a) essential amino acid composition, (b) amino acid digestibility (bioavailability), (c) amino acid requirements of the species consuming the protein (biological value of proteins), and (d) the ability to use the protein for growth (protein efficiency ratio) (Eggum 1985). Eggum (1970) has determined that lysine contained in globulin proteins, which is present in the aleurone layer, has the lowest nitrogen digestibility because enzymes are not able to penetrate the globular structure and liberate the amino acids. Glutamic acid, which is largely present in the prolamin fraction of the endosperm, is highly digestible and all other amino acid digestibility values fall within these two extremes. As shown in Table 2.7, nitrogen digestibility values for casein, wheat, soybean, and animal products are the highest. Even though the nitrogen digestibility value for barley is somewhat lower, it is still comparable to that of eggs and other cereals.

Table 2.6. Amino acid composition (g/16 g N) of regular barley proteins[1].

Amino acid[2]	**Whole grain**	**Protein fraction**			
		Albumin	**Globulin**	**Hordein**	**Glutelin**
*Lysine	3.5	5.1	5.3	0.5	2.2
Histidine	2.2	1.9	1.7	1.0	1.9
Arginine	4.8	3.8	5.5	2.0	3.2
Aspartic acid	5.3	10.4	8.1	1.6	4.2
*Threonine	3.3	4.6	4.4	2.0	3.8
Serine	4.2	5.4	5.4	4.2	5.7
Glutamic acid	24.4	14.5	15.5	34.7	25.9
Proline	11.6	7.3	9.3	23.0	14.1
Glycine	3.8	8.9	9.6	2.5	6.3
Alanine	3.6	10.6	7.9	2.4	5.1
Cysteine	2.1	0.5	0.0	1.6	0.5
*Valine	5.3	7.2	7.6	4.6	6.5
*Methionine	1.7	1.9	1.8	0.9	1.3
*Isoleucine	3.7	4.1	3.8	3.8	4.2
*Leucine	7.2	7.9	7.9	6.9	8.2
Tyrosine	3.5	2.8	2.8	2.4	2.7
*Phenylalanine	5.3	3.6	3.6	5.9	4.3
Tryptophan	1.3	-	-	-	-

*Essential amino acids.

[1] Adapted from Lasztity (1984).

- values not reported.

Table 2.7. Nitrogen digestibility values of some proteins[1].

Source	**Nitrogen digestibility (%)**[2]
Casein	99
Wheat	92
Corn	90
Soybean meal	90
Beef	90
Pork	89
Egg	88
Barley	82
Rye	81
Oats	79

[1] Adapted from Bodwell (1985).

[2] (Total nitrogen intake/total nitrogen remaining at the end of the small intestine) x 100%.

In Table 2.8, the biological values (BV) and protein efficiency ratios (PER) are given for several grains and eggs (used as a standard). When compared to other cereal grains, barley has a high BV and PER and combined with an 82% nitrogen digestibility value, barley makes for an ideal feed or food source for both animals and humans. As well, with the development of high-protein and high-lysine barley varieties (i.e. Hiproly), any nutritional deficiencies due to the low levels of lysine and methionine can be eliminated.

The carbohydrate content of barley directly influences the nutritive quality of proteins. According to Newman and Newman (1989), low to medium levels of proteins are more desirable to ensure optimum protein and starch bioavailability. Protein levels of about 12% seem adequate because higher protein concentration leads to a decrease in starch content. Also, unless one is dealing with a high-lysine variety, any increase in protein is due to increased hordein synthesis in the endosperm. Hordeins are low in essential amino acids, which contribute to their low nutritional value.

On average, proteins provide 12-16% of the total daily energy intake, while the value for carbohydrates is 52-56%. Not only do carbohydrates provide energy for protein metabolism, but they are also responsible for supplying all the energy needs related to metabolic, kinetic and thermal needs of the body (Vanicek and Turek 1985). Thus, if proteins are involved in glucogenesis, they lose their biological value because the amino acids are used to make glucose for energy needs instead of biosynthesis of nitrogenous compounds. Therefore, carbohydrates and proteins should be used together to obtain their most favorable dietary value (Mahan and Escott-Stump 2000).

Table 2.8. Protein quality of cereal proteins[1].

Source	BV[2]	PER[3]
Wheat	58-67	0.9-1.7
Oat	74	2.0-2.5
Barley	75	1.6-2.0
Corn	53-60	0.9-1.3
Rye	74	1.8-2.3
Soy isolate	66	1.6
Egg	95	3.5

[1] Adapted from Nierle (1985).
[2] BV = biological value (retained nitrogen/absorbed nitrogen) x 100%.
[3] Protein efficiency ratio = weight gain by animal/amount of protein fed.

The protein requirements for all animals are not the same. Barley proteins can be used as the sole feed grain in pigs and cattle, but since the lysine requirement for poultry and humans is somewhat higher, barley feed must be supplemented with other grains and oilseeds such as oat and soybean meal for it to be effective (Hickling 1993). Helm (1992) suggests that both plant breeders and nutritionists have to combine their scientific knowledge to enhance the utilization of barley as a high value food/feed source.

2.2.4. Protein extraction

Through plant breeding and genetic engineering, cereal proteins have the potential of satisfying nutritional needs as per meat products (Pomeranz 1987), but research in plant genetics in terms of modifying the amino acid composition is limited. The North American and European diets rely heavily on meat-based products; therefore, the necessity of cereals as a protein source is not very crucial as it is in third world countries. Nevertheless, methods such as extraction, chromatography, electrophoresis and amino acid sequencing have been developed to quantify, isolate and characterize protein fractions (Bietz 1979). Extraction has been used to concentrate cereal proteins to determine their nutritional and functional properties. Isolation of wheat proteins (gluten) has led to its widespread use in baked products, whereas meat analogs have been successfully manufactured from soy proteins.

2.2.4.1. Protein separation according to Osborne solubility classes

In 1924, Osborne was the first to classify wheat proteins based upon their solubility in various solvents (Shewry et al 1978). Albumins (water soluble) and globulins (soluble in salt solutions) are extracted together in salt solutions followed by dialysis. Prolamins are isolated using alcohol and the remaining glutelins are obtained under alkali conditions. But due to the complexity of the other cereal proteins, there is considerable overlap and contamination between cytoplasmic and storage proteins. Since then, other scientists have modified the original Osborne technique in an attempt to obtain proteins of higher purity.

Proteins are isolated and purified for the purpose of characterization based upon their shape, size and molecular weight. Shewry et al. (1978) evaluated several techniques for the separation of barley storage proteins. Hordein fraction was obtained using 55% (w/w) propan-2-ol plus 2% (w/w) 2-mercaptoethanol at 60°C and glutelins were obtained using sodium dodecyl sulfate (SDS) and 0.6% (w/w) 2-mercaptoethanol at pH 10. By using SDS polyacrylamide gel electrophoresis, protein fractions can be fingerprinted through determination of apparent molecular weight, which can be used for varietal identification. Shewry et al (1977) identified three classes of hordeins (A, B and C) based upon their molecular weight with each fraction having a different amino acid composition. Fractions A (13-20 kilodaltons (kDa)) and B (30-51 kDa) were low in lysine, glutamine and proline, whereas fraction C (67-86 kDa) had trace lysine but very high levels of glutamine and proline. Fractions A and C were readily extracted by alcohol alone, but fraction B was only partially soluble in alcohol and therefore a combination of elevated temperatures and reducing agents must be used.

Singh and Sastry (1977) partially isolated hordeins using 70% ethanol but were unsuccessful in separating glutelins using alkali solution. Glutelins form polymers of high molecular weight (20-68 kDa) through extensive cross-linking of disulfide bonds, thus the isolation of glutelins requires them to be reduced and then alkylated. Wilson (1981) reported that even though alkylation of glutelins gives better resolution of polypeptides, this step can be omitted because extraction of glutelins using reducing agents such as mercaptoethanol, urea and dithiothreitol is just as effective. Glutelin extraction is frequently affected by how efficiently the hordeins have been removed. Most often, glutelin fractions are contaminated by the presence of hordein polymers due to incomplete extraction. Glutelins appear to be composed of two major protein types; one with an amino acid composition resembling that of albumins and globulins and the other with an amino acid profile similar to that of hordeins (Landry et al 1972).

Methods of extraction of albumins and globulins (10-70 kDa) are simpler than those used for storage proteins. Salt solutions of 0.5M NaCl, either at room temperature or 4°C, with or without the addition of sodium ethylenediaminetetraacetic acid (Na-EDTA), is most commonly used. Following dialysis, the albumin remains in solution while globulins precipitate out (Koie and Nielsen 1977). Essential amino acids, lysine, threonine, methionine and valine are present at a higher level in the salt-soluble fraction as compared to the whole seed. Rhodes and Gill (1980) compared the quality of salt-soluble proteins in normal barley versus high-lysine types. In normal barley, albumins and globulins together accounted for 11-18% of total proteins, whereas in high-lysine varieties, this figure rose to 18-33%. Ingversen and Koie (1973) determined that, of the total lysine content, water- and salt-soluble proteins accounted for almost 50% of lysine, with the value being generally higher in the globulin fraction as compared to that in the albumin fraction (Rhodes and Gill 1980).

Due to the complexity of barley proteins, the classical Osborne type extractions cannot be used directly (Shewry et al 1978). Various adjustments have to be made (especially for the isolation of storage proteins) to obtain purified protein fractions. Nevertheless, the Osborne procedure has led to the classification, quantification and nutritional evaluation of cereal proteins that give scientists a better understanding and knowledge of how proteins behave in complex food systems.

2.2.4.2. Alkali extraction

Alkali extraction is a method used to isolate proteins from cereal flours and oilseeds. The efficiency of extraction is based upon solvent type, concentration, solubilization pH and isoelectric pH to precipitate out proteins. Generally, for cereal grains, the common solvent used is $0.03M$-$1.0M$ NaOH, solubility pH is between 9.5-12, isoelectric pH ranges from 4.0-6.0 with a solvent-to-feed ratio between 5:1 to 10:1. Each of these parameters are species specific, such that one given set of conditions will not effectively extract proteins from every cereal grain. Reports on alkali extraction of cereal protein concentrates include oats (Cluskey et al 1973; Wu et al 1973)), wheat (Wu and Sexson 1975), corn (Wu and Sexson 1976a,b), triticale (Wu et al 1976), sorghum (Wu 1978), barley (Wu et al 1979) and wheat germ (Hettiarachchy et al 1996).

Protein concentrates obtained by alkali extraction typically have high extractability and purity and are higher in essential amino acids as compared to the feed flour. They are low in starch, fat and ash and have a bland flavor (Wu et al 1976). Wu and Sexson (1975) postulated that a high percentage of alkali-extracted proteins are salt-soluble proteins (albumins and globulins) and glutelins. Ultimately, the functionality of protein concentrates in food products depends on the extraction conditions employed and the compositional profile of the extracted proteins.

2.2.5. Functional properties

Due to the socio-economic conditions, the lifestyles of consumers have changed drastically over the past 10-15 years. People are leading a fast-paced life; gone are the days where the kitchen table was a means of social interaction. The consumer has become highly demanding of food products that complement their busy lifestyle. Food has to be convenient, nutritious, safe and has to taste good. This increasing trend in consumer expectations has led to food manufacturing companies constantly finding new ways to enhance food quality attributes (i.e. flavor, color, odor, taste, texture and mouth feel) to satisfy consumer needs.

The term "functional property" is defined as the chemical and physical attributes of an ingredient, which affect a given food system during processing, storage and consumption (Kinsella 1981). In essence, functional properties affect the foods' physical and sensory traits. Proteins are an important class of compounds because they are stable during various processing conditions, form network structures, interact with other food components and provide essential amino acids (Phillips et al 1994). Proteins are an integral part of several major foods, such as milk, cheese, meat, egg, bread and other baked goods. Functional properties of proteins are governed by their intrinsic characteristics. Factors such as protein structure (size and shape), charge distribution (polar or neutral) and presence of intermolecular and intramolecular bonding of specific chemical groups (Pomeranz 1991) all play an important role in the behavior of proteins in the presence of other food components. The challenge for food scientists is to find proteins that will possess multiple functionality or excel in a particular area as highlighted in Table 2.9.

Protein solubility is a very important functionality for beverage systems and is dependent on the polar nature of the protein, which is affected by pH. Under acidic conditions, the carboxyl group is protonated to COOH and the protein exists as a cation due to the formation of NH_3^+. Under basic conditions, the NH_3^+ is deprotonated to NH_2 and the protein primarily exists as an anion due to the formation of COO^-. Therefore, due to the presence of charged groups, water solubility is highest because surface area for water binding increases as well as the repulsion forces between similarly charged protein molecules (Wall 1979). Water binding capacity is the ability of a protein to bind water within a protein matrix, which includes both bound water and entrapped water (Kinsella 1981). Water holding capacity of a protein contributes to moistness/juiciness attribute in foods such as meats and baked goods (i.e. breads, cakes and muffins). Proteins with amino acids containing polar (cationic, anionic, polar neutral) side chains bind 4-7 molecules of water while hydrocarbons (non-polar) bind only a single molecule and hydrophobic groups bind very little to none (Zayas 1997).

Table 2.9. Basic functional properties performed by proteins in food systems[1].

Functional property	Mode of action	Food system
Solubility	Protein solvation	Beverages
Water absorption and binding	Hydrogen binding of water, Entrapment of water (no drip)	Meat, sausages, breads and cakes
Viscosity	Thickening, water binding	Soups and gravies
Gelation	Protein matrix formation and setting	Meats, curds and cheese
Cohesion-adhesion	Protein acts as adhesive material	Meat, sausages, baked goods and pasta products
Elasticity	Hydrophobic bonding in gluten, disulfide links in gels	Meats and bakery
Emulsification	Formation and stabilization of fat emulsions	Sausages, bologna, soup and cakes
Fat absorption	Binding of free fat	Meats, sausages and doughnuts
Flavor-binding	Adsorption, entrapment and release of flavor compounds	Simulated meats, baked foods
Foaming	Form stable films to entrap gas	Whipped toppings, chiffon desserts and angel cakes

[1] Adapted from Kinsella (1981).

Proteins are important emulsifiers because they possess the ability to disperse oil and keep oil droplets suspended in the water phase without causing seperation (oil-in-water (o/w) emulsion). Emulsifying properties of a protein is dependent on its hydrophobicity and hydrophilic-lipophilic balance (Zayas 1997). Proteins that contain a high percentage (>30%) of non-polar amino acid residues exhibit good emulsion and foaming properties (Nakai 1983). Proteins have amphiphilic characteristics, thus the more hydrophobic the protein, the lower is the interfacial tension between water and oil, resulting in a more stable emulsion (Nakai et al 1980).

In essence, foaming properties of proteins are similar to those found for emulsions. The basic mechanism of foam formation, using proteins as surfactants is the ability of proteins to uncoil and interact with both water and air. The proteins are adsorbed at the water-air interface forming a film, which entraps air, thus forming bubbles. Then, as the protein films surrounding the air bubbles get closer, the flow of liquid is restricted, leading to decreased drainage and increased foam stability (Cherry and McWatters 1981). The effectiveness of a protein to form both a foam and an emulsion depends on several of its inherent properties. Proteins should have the ability to solubilize in various solvent systems, denature to form a cohesive monolayer of film at the different interfaces and maintain an optimal ratio between hydrophilic and hydrophobic interactions to prevent protein aggregation and precipitation, leading to phase instability (Kinsella 1976).

The main functional properties such as solubility, water holding capacity, viscosity, gelation, fat binding capacity and foam and emulsion formation are all dependent on the chemical nature of the side chains of the amino acids making up the protein network. Due to the heterogeneous structure of proteins and their ability to interact with other food components (i.e. oil, sugar and water), they display a wide range of functionality.

To date, most of the protein functionality research has focused on milk, egg and meat proteins. In terms of cereals and legumes, attention has been given mainly to soybean proteins and gluten proteins of wheat. Examination of proteins of other cereals such as barley is needed as they may impart both nutritional and functional properties, which may be more effective and economical as food components as compared to other protein ingredients.

2.3. OTHER SOURCES OF PROTEIN INGREDIENTS

Total world cereal grain production for the year 2002 was 2000 Mt, the highest for any food commodity and wheat, rice and corn accounted for 88% of total cereal production (Table 2.10). World production of barley was similar to, but lower than that for soybeans but higher than oats, beans, lentils whey, beef, chicken and eggs. The daily protein consumption from each food commodity is also highlighted in Table 2.10. Protein intake from cereals (23-32 g) was the highest among all food sources in the world, North America and Canada and wheat alone accounted for 82-88% of total protein intake from cereals.

In North America and Canada, most protein intake comes from wheat, beef and chicken as compared to the world, but the world consumption of rice is substantially higher as compared to that in North America and Canada. Event though the protein intake from soybean in North America and Canada is low (0.1-0.6 g/person/day), the sales of soy based products in North America was expected to reach $314 million in the year 2002 (Chapman 1998).

Table 2.10. World production of some major protein-based food sources and daily protein consumption.

Food source	% Protein[1]	Production[2] (Mt)[3]	Daily intake[4] of protein/person/ day (g)	Daily intake[4] of protein/person/ day (g)	Daily intake[4] of protein/person/ day (g)
			World	North America	Canada
Cereals (Total)	6-18	2,000	32.3	25.1	23.6
Wheat	6-16	573	15.7	20.5	21.0
Barley	8-16	132	0.2	0.2	0.10
Oat	9-16	25	0.1	0.7	0.30
Corn	8-10	602	3.6	1.7	0.40
Rice	7-8	576	10.5	1.7	1.4
Beans	25-27	18	1.2	1.9	0.9
Lentils	20-22	3	-	-	-
Soybean	40-50	180	2.5	0.1	0.6
Whole cow milk	3-4	502	4.1	10.2	5.0
Dry Whey	70-72	2	0.0	0.3	0.3
Beef	17-18	61	3.6	14.4	12.2
Chicken	31-32	63	3.9	16.8	13.0
Eggs	11-12	58	2.5	4.2	3.5

[1] Bodwell (1985).
[2] Adapted from FAO (2002).
[3] Mt = Million tonnes
[4] Adapted from FAO (2001).

Based upon these statistics, it appears that there is still a tremendous potential for protein-based food ingredients from cereal grains (such as barley) to break into the world market. For a protein ingredient to be successful, it must possess nutritive value, high solubility and must be free of adverse flavors and there must be the potential for high production capacity in an economically feasible manner.

The scope of this thesis focuses on the extraction and functionality of barley proteins and its by-products (β-glucan and starch). Properties of wheat and soybean proteins are discussed in this section since they share a substantial portion of the food protein ingredient market (Table 2.10). In order for barley proteins to be successful as a food ingredient, one has to evaluate and understand how the functionality of barley proteins compares to that of wheat and soy proteins and thus assess the acceptability of such products.

2.3.1. Wheat protein structure and function

From Tables 2.1 and 2.2 it is quite evident that wheat production in Canada dominants other cereal grains. However, protein intake from wheat in Canada, North America and the world is highest among all other protein sources (Table 2-10). Wheat is grown, in various amounts, in all parts of the world and serves as a major source of carbohydrates and protein. A typical wheat grain is comprised of approximately 60-68% starch, 7-18% protein, 1.5-2% ash and 1.5-2% fibre (Matz 1991). In wheat, the proteins, lipids and sugars are mostly present in the germ, whereas the pentosans and ash are concentrated in the bran and starch is highly localized in the endosperm (Pomeranz 1991).

Compared to barley, wheat proteins are higher in albumins (5-10%) and prolamins (40-50%) but lower in globulins (5-10%) and glutelins (30-40%) (Pomeranz 1987). During processing of wheat, 72-75% of the grain is milled to obtain white flour, which mainly consists of starch and proteins. The protein content in milled flour is 12-13% (w/w) and accounts for 91-92% of the total proteins in the wheat grain and mainly comprises of prolamins (gliadin) and glutelins (glutenin), which combine to form the gluten (Pomeranz 1971). Gluten is the strong viscous dough matrix, which remains behind once the starch and other soluble material is washed away and has the ability to retain gas and stretch during leavening of baked products such as breads, cakes and muffins. Properties of gluten are determined by the functional properties of endosperm proteins, gliadin (very cohesive with low elasticity) and glutenin (elastic with little cohesiveness).

Gluten protein complex is high in glutamic acid, proline, cysteine and cystine and low in basic amino acids (lysine, arginine and histidine) (Lasztity 1984). Almost all of the glutamic acid exists in its amide form of glutamine and combined with the low content of basic amino acids, gluten proteins have a low charge density at neutral pH values. The low charge density decreases the repulsion forces and allows the polypeptide chains to interact with each other through hydrogen, disulfide and dipolar interactions (Hoseney 1994). In addition, Shewry et al (1994) have determined through amino acid sequencing that high molecular weight subunits of glutenin contain cysteine (sulfur containing amino acid) at both the N- and C-terminals of the protein chain. This aspect allows for crosslinks at both ends forming a network structure through disulfide bonds to adjacent glutenin polypeptides. In glutenin proteins, the C- and N-terminals are responsible for the α-helix structure due to the cysteine residues and non-repeating amino acid sequences at both ends of the protein chain. The central section of the protein molecule contains very little to no cysteine, but is characterized by both interspersed and randomly repeating subunits of amino acids, which make up the β-turn structure (Lasztity 1984; Shewry et al 1994). Starch and gliadin are dispersed throughout the glutenin matrix; therefore, the amino acid content and sequencing pattern of glutenin proteins are very important in the formation of wheat dough.

2.3.2. Soybean protein structure and function

Use of soybeans as a food source has been reported back in the 11th century in Northern China (Wolf 1981). Consumption of soybeans has been restricted to the eastern part of the world and saw little use in the Western Hemisphere. Soybean was first introduced to the U.S. in the late 1920's, and was processed to make edible oil for human use and defatted meal for the livestock industry. Since then, the market has seen the introduction of numerous soybean products including, but not limited to, tofu (soybean curd), milk, oil, texturized meat analog proteins (i.e. wieners, patties, bacon bits, and meatballs) and soybean flour for use in baked products and infant formulas. Overall aspects of soybean protein functionality in terms of mimicking meat like products will be examined next.

Table 2.11 shows the proximate analysis of flour and various protein components of soybean. Proteins of soybeans are separated into three groups based upon function. Metabolic and structural proteins maintain normal cellular activity while the storage proteins provide a source of nitrogen and carbon for the seed during germination and growth (Wolf and Cowan 1975). Storage proteins account for 80-90% of total soybean protein content and are made up of globulins, which exist as protein bodies in the cotyledon and hypocotyl tissue of the oilseed (Pomeranz 1991).

The two major globular proteins that exist in soybeans and contribute to the formation of a protein matrix or network similar to that of gluten proteins of wheat are 7S (β- and γ-conglycinin) and 11S (glycinin) proteins. Nomenclature of soybean proteins is based upon their sedimentation coefficient, which is correlated to their molecular weight. Molecular weight of 7S globulins are in the range of 105-200,000 Da whereas the 11S fraction is almost twice as large with a molecular weight range of 300-350,000 Da (Garcia et al 1997).

Conglycinin (7S) is a quaternary glycoprotein with 4% carbohydrate (glucose and mannose) content. It contains both polar and non-polar amino acids resulting in significant hydrophilic and hydrophobic interactions. Glycinin (11S) contains both acidic and basic amino acids as well as higher concentrations of sulfur due to the presence of cysteine and methionine. As a result, glycinin has the ability to form larger stable aggregates through extensive disulfide and sulfhydryl linkages, which have a higher water holding capacity and tensile strength (Hermansson 1978).

Table 2.11. Proximate composition (% w/w) of soybeans[1], flour[2] and protein fractions[2].

Proximate Analysis	Whole soybean	Flour and protein type		
		Defatted flour	Protein concentrate	Protein isolate
Moisture	-	6.5	4.9	4.7
Protein	40.0	53.0	68.0	92.0
Carbohydrate	34.0	31.0	19.0	-
Fat	21.0	4.0	0.3	-
Ash	4.9	6.0	5.0	3.4
Fibre	1.1	2.5	2.6	0.1

[1] Adapted from Wolf and Cowan (1975). Values are on a moisture-free basis.
[2] Adapted from Smith and Circle (1978).

The two main processes used to obtain meat analog products are extrusion and spinning. Extrusion involves the use of high pressure and high temperature (690 kPa and 150-180°C) cooking, which causes protein denaturation to smaller subunits forming a viscoelastic protein mass (Campbell 1981). Reformation of aggregates occurs mainly due to intermolecular amide bonds, which may cause some linear alignment of high molecular weight proteins (11S and 15S), but not to the extent seen in spinning. Therefore, this fibrous network system has high porosity and hydration capacity forming a moist, soft and chewy product (Cambell 1981). The starting material for extrusion can be either flour (whole or defatted), protein concentrate or isolate whereas for spun fibres, only soy protein isolate is utilized due to its high protein purity.

Spinning is done under alkaline conditions (pH 10-11) at temperatures of 40-50°C. Alkalinity causes unfolding of protein chains, facilitating linear sulfhydryl/disulfide bonding and acidification causes aggregation of linear polypeptides through hydrogen and ionic bonding. The final step of towing and stretching causes further linear association along the protein axis leading to fibre formation (Wolf 1970). In contrast to extrusion, spun products are dry, fibrous and chewy and are mainly formed into granules or imitation bacon bits as used in many soup products.

In both processing techniques used, operational parameters, such as pH, salt concentration (ionic strength), temperature, pressure, and solvent type affect the conformational structure of soybean proteins. In terms of meat analog formation, proteins must be of high molecular weight, maintain linear symmetry without the presence of bulky side chains, which may lead to steric hindrance and have a high content of polar groups and sulfur content for ionic, hydrogen and covalent bonding (De Valle 1981). By manipulating and optimizing extraction conditions and processing methods, meat imitation products utilizing soybean proteins of varying sensory qualities can be achieved.

In October 1999, the U.S. Food and Drug Administration (FDA) approved a health claim stating that, "diets low in saturated fat and cholesterol that include 25 grams of soy protein a day may reduce the risk of heart disease". It was shown in 27 clinical studies that soy proteins have the ability to reduce total cholesterol and low density lipoproteins (LDL) by up to 8% (Henkel 2000). Isoflavones, a plant estrogen, are also present in soybeans and depending on the extent of processing, isoflavones can be in the range of 7.01 mg in soy milk, 24.7 mg in soy tofu and 128.3 mg in roasted soy nuts. Studies have shown that isoflavones prevent the formation of plaque, caused by LDL cholesterol, on the walls of blood vessels (Koszewski and Jones 2002). The research on health benefits of isoflavones are mixed, because scientific studies have indicated that consuming over 40 g of soy a day has been linked to breast cancer in postmenopausal women (Atkinson and Bingham 2002; Koszewski and Jones 2002) while other studies have indicated just the opposite (Yamamoto et al 2003). Furthermore, isoflavones such as genistein and daidzein, have been shown to interfere with thyroid function but no such findings have been reported for soy proteins for which the FDA health claim was based upon (Henkel 2000; Koszewski and Jones 2002).

There are numerous wheat and soybean based products on the food market today. Barley has a better nutritional value than wheat and may compete with soybean products in terms of taste and food safety due to the absence of isoflavones. Processing of soybean components under extreme pH and temperature conditions has led to the destruction of methionine (limiting amino acid) and cysteine and formation of toxic dipeptides such as lysinoalanine, which has been shown to cause lesions in the kidneys of rats (Wolf 1981; Liu 1999). Furthermore, Wolf (1981) and Liu (1999) have indicated that improper deactivation of trypsin inhibitors in soybean has resulted in pancreas enlargement in experimental animals (Wolf 1981; Liu 1999). In terms of taste, soybean products have a "beany flavor", which is regarded as an undesirable attribute by consumers. Thus, if barley products can take advantage of or compete against some of the negative aspects related to wheat and soybean, then perhaps a potential market may exist for barley-based protein products.

2.4. BARLEY β-GLUCAN

In barley, β-glucan is a soluble dietary component and is the third highest component (4-8%) only after starch and protein. Dietary fibre has been shown to have various therapeutic effects, including lowering cholesterol, modulating blood glucose levels in diabetics and preventing the onset of coronary heart disease and colon cancer (Glicksman 1986). Therefore, to have an optimum effect, a combination of soluble (i.e. β-glucan) and insoluble (i.e. cellulose) fibre should be included in the diet as they contribute differently to maintaining proper health. As recommended by various health organizations, 20-35 g of dietary fibre should be consumed per day (Hudson et al 1992).

2.4.1. Structure and function

In barley, (1→3),(1→4)-β-D-glucan (β-glucan) accounts for 70-75% of the endosperm cell wall content while arabinoxylans, glucomannans, cellulose and phenolic compounds make up the rest of the cell wall components (Newman et al 1989). β-Glucan is a non-starchy polysaccharide made up of a linear chain of glucose molecules containing approximately in a 1:3 ratio of β-(1→3) and β-(1→4) links. Based upon the structure of β-glucan, its molecular weight range and axial ratio, Woodward et al (1983) postulated that β-glucan is a flexible molecule that assumes a worm-like shape in aqueous solutions. The irregular configuration of β-glucan is attributed to the random occurrence of β-(1→3) bonds, which creates kinks in the molecule thus enabling it to solubilize in water.

2.4.2. Health benefits of barley β-glucan

Barley β-glucan (BBG) has received considerable attention in the past 10-15 years due to its potential health benefits. It has been demonstrated that in male diabetic patients, the soluble and viscous nature of β-glucan in the digestive tract delays the release of glucose in the blood following a meal, thus curtailing the need for insulin (Wood 1984). Kahlon et al (1993) performed a clinical study to compare cholesterol-lowering effects in male hamsters by using various concentrations of BBG enriched fractions, rice bran and oat bran. This study showed that diets comprising of 6% (w/w) BBG lowered total cholesterol by 15%, which was the highest value among all treatments studied. In a similar study carried out by Klopfenstein (1988), breads containing 7% (w/w) BBG reduced overall serum and liver cholesterol levels in rats. BBG also selectively decreased the level of LDL that is a major contributor to plaque build up in the arteries, while not disturbing the concentration of high density lipoproteins (HDL), which guard against atherosclerosis. Newman et al (1989) showed that when adult human subjects (both male and female) consumed 42 g of barley dietary fibre over a 28 day period, their serum cholesterol and LDL levels decreased by 5% and 14%, respectively, and surprisingly, their HDL levels increased by 10%.

High glycemic index (GI) foods have been positively associated with causing the increased risk of type 2 diabetes and coronary heart disease (Jenkins et al 2002). A breakfast cereal enriched with 8.1% (w/w) oat β-glucan and food bar enriched with 6.5% (w/w) oat β-glucan was fed to sixteen human subjects with type 2 diabetes. The results showed that oat β-glucan fortified diets had 43-65% lower GI values (determined 3 hr after consuming the meal) as compared to diets comprised of white wheat bread and a commercial oat bran cereal (Jenkins et al 2002). Cavallero et al (2002) designed a study to compare the GI values of white wheat bread compared to bread consisting of 80% white wheat flour and 20% barley flour, containing 6.3% BBG with 90% solubility. When comparing the GI (2 hr after the meal was consumed) values, the six human subjects consuming the BBG enriched bread had 28% lower GI value as compared to the white wheat bread whose GI value was 89. Therefore, BBG can be potentially used in foods with a high GI as its viscous nature will slow down the release of glucose in the blood stream following a meal.

The compatibility of β-glucan in different food systems is very important in determining its potential use commercially. Temelli (1997) has observed that the isolation of β-glucan is significantly affected by extraction conditions such as temperature, pH and temperature*pH interactions. Thus, depending on the extraction procedure used, variation does exist in the final product obtained in terms of β-glucan recovery, purity and its functional properties especially viscosity, which has been attributed to causing the health benefits of barley. Therefore, treatments can be manipulated to obtain a BBG fraction suitable for a specific purpose within a given food system.

2.5. BARLEY STARCH

Starch is the largest component in the barley grain. Found in the endosperm, it represents 65-75% (based on variety) of the dry kernel weight. The carbohydrate content of barley provides an excellent source of energy. Yet, barley finds use as a human food commodity to a minimal extent simply because it is seen as a feed grain and not utilized in further processing (Czuchajowska et al 1998). As experimental data accumulate on the structure and functionality of barley starch (in comparison to wheat, corn, and potato starches), its potential use in non-malting food applications is becoming promising.

2.5.1. Structure and function

Barley starch contains both large and small granules, which are classified as A-and B-type, respectively. The larger granules range in diameter from 15-25 µm whereas the smaller irregular ones are usually less than 10 µm in size (MacGregor and Fincher 1993). In proportion to the total number of starch granules, A-type constitutes only 10-20% while representing 85-95% of the total weight of starch (Morrison et al 1986). Vasanthan and Bhatty (1996) studied physicochemical properties of small and large starch granules in different barley varieties. The results indicated that there was a greater difference among genotypes than between the bimodal starch granules within the same genotype. But, Myllarinen et al (1998) have shown that, as compared to large granules, small granules contain more lipid-complexed amylose, which affects swelling, solubility and gelatinization properties. Thus, understanding the characteristics of a particular starch type is very important in terms of its behavior in a particular food system, as in the case of malting and brewing.

Based upon the amylose and amylopectin contents, barley flours and starches are placed in three basic categories. These include, high-amylose (amylose content > 40%), normal (amylose content of 25-30%) and waxy (amylose content of < 5%) varieties (Bhatty 1999). The structures of both of these major components of starch are quite different, which lead to their unique behavior. Amylose is a linear molecule containing long chains (average chain length of 1,800 anyhydro-glucose units) of α-(1-4) linked D-glucose residues. Amylopectin also contains α-(1-4) linked D-glucose residues, but in addition this linear arrangement is interrupted by α-(1-6) linked D-glucose fragments, which results in extensive branching. As compared to amylose, amylopectin chain length is quite small (average length is 21-25 anhydro-glucose units) but the molecular weight of amylopectin is approximately 15 times higher (MacGregor and Fincher 1993). Therefore, waxy barley starches exhibit high swelling power and colloidal stability while high amylose starches are more suitable in systems where gel- or film-forming properties are needed (Bhatty 1993).

2.5.2 Barley flour and starch end uses

Berglund et al (1992) conducted extensive studies on the food uses of waxy hull-less barley flour. Products included muffins, breads, cookies, biscuits and noodles. Based on the type of product formulated, barley flour was substituted between 25-100% for whole wheat flour. In general, Berglund et al (1992) concluded that barley flour products were liked as well as other products containing wheat, oat or rice. The exception was that it was not possible to use 100% barley flour to produce commercial bread due to gumminess, leading to a less-cohesive structure. But, in an earlier study carried out by Swanson and Penfield (1988), a successful formula for whole grain bread was developed using 20% barley flour and 30% whole wheat flour. Compared to 100% whole wheat bread, the sensory (appearance, texture and flavor) results were favorable for this type of product.

Pita bread (Arabic bread), which is a type of flat bread, is a major part of the carbohydrate (along with rice) diet in the middle eastern countries. Toufeili (1999) formulated pita breads using starches from wheat, barley, corn, oat, potato, rice and sorghum. In terms of textural attributes (tearing, cohesiveness and rollability), there was no significant difference ($P < 0.05$) between pita breads made from wheat or waxy barley starch. However, pita breads prepared with barley starch staled at a much faster rate as compared to wheat. In part, this effect was linked to the retrogradation of amylopectin, which is proportionally higher in waxy barley starch. By increasing the cross-links between amylopectin and phosphorous oxychloride, the rate of staling was decreased because of restriction to starch swelling caused by the cross-links. In retrospect, as knowledge of barley starch structure, chemistry and physiological properties become more understood, this will aid in the increased utilization of barley in malting, food and industrial applications.

2.6. REFERENCES

Atkinson C., and Bingham, S.A. 2002. Mammographic breast density as a biomarker of effects of isoflavones on the female breast. Breast Cancer Res. 4: 1-4.

Atkinson, G. 2003. September estimate of production of 2003 principle field crops, Alberta. At http://www1.agric.gov.ab.ca/$department/deptdocs.nsf/all/sdd7533?opendocument (accessed on October 28, 2003).

Bamforth, C.W., and Barclay, A.H.P. 1993. Malting technology and the uses of malt. Ch. 7 in *Barley Chemistry and Technology*, A.W. MacGregor and R.S. Bhatty (Ed.), p. 297-354. American Association of Cereal Chemists, Inc., St. Paul, Minnesota.

Bergland, P.T., Fastnaught, C.E., and Holm, E.T. 1992. Food uses of waxy hull-less barley. Cereal Foods World. 37(9): 707-714.

Bhatty, R.S. 1986. The potential of hull-less barley-A review. Cereal Chem. 63(2): 97-103.

Bhatty, R.S. 1993. Nonmalting uses of barley. Ch. 8 in *Barley: Chemistry and Technology*, A.W. MacGregor and R.S. Bhatty (Ed.), p. 355-417. The American Association of Cereal Chemists, Inc., St. Paul, Minnesota.

Bhatty, R.S. 1999. The potential of hull-less barely. Cereal Chem. 76(5): 589-599.

Bietz, J.A. 1979. Recent advances in the isolation and characterization of cereal proteins. Cereal Foods World. 24(5): 199-207.

Bodwell, C.E. 1985. Amino acid content as an estimate of protein quality for humans. Ch. 1 in *Digestibility and Amino Acid Availability in Cereals and Oilseeds*, J.W. Finely and D.T. Hopkins (Ed.), p. 1-14. American Association of Cereal Chemists, Inc., St. Paul, Minnesota.

Campbell, M.F. 1981. Processing and product characteristics for textured soy flours, concentrates and isolates. J. Am. Oil Chem. Soc. 58: 336-338.

Cavallero, A., Empilli, S., Bringhenti, F., and Stanca, A.M. 2002. High (1 →3, 1 → 4)-β-glucan barley fractions in bread making and their effects on human glycemic response. J. Cereal Sci. 36: 59-66.

Chapman, N. 1998. Third annual soyfoods symposium proceedings: Where is the soyfood market headed? At http://www.soyfoods.com/symposium98/ChapmanPaper98.html (accessed on October 4, 2003)

Chaudhari, R. 1997. Foods of the future: The impact of functional foods in the cereal industry. Cereal Foods World. 5: 94-95.

Cherry, J.P., and McWatters, K.H. 1981. Whippability and aeration. Ch. 8 in *Protein Functionality in Foods*, J.P. Cherry (Ed.), p. 149-176. American Chemical Society, Washington, D.C.

Cluskey, J.E., Wu, Y.V., Wall, J.S., and Inglett, G.E. 1973. Oat protein concentrates from a wet milling process: Preparation. Cereal Chem. 50: 475-481.

Conrad, S. 1999. Grains in the canadian diet. National Institute of Nutrition. 14(2): 1-4.

Czuchajowska, Z., Klamczynski, A., Paszczynska, B., and Baik, B.K. 1998. Structure and functionality of barley starches. Cereal Chem. 75(5): 747-754.

Del Valle, F.R. 1981. Nutritional qualities of soya protein as affected by processing. J. Am. Oil Chem. Soc. 58: 419-429.

deMan, J.M. 1999. Proteins. Ch. 3 in *Principles of Food Chemistry*, 3rd edition, J.M. deMan (Ed.), p. 111-162. Aspen Publications, Gaithersburg, Marylamd.

Duffus, C.M., and Cochrane, M.P. 1993. Formation of the barley grain-Morphology, Physiology, and Biochemistry. Ch. 2 in *Barley: Chemistry and Technology*, A.W. MacGregor and R.S. Bhatty (Ed.), p. 31-72. The American Association of Cereal Chemists, Inc., St. Paul, Minnesota.

Egum, B.O. 1970. Nutritional evaluation of proteins by laboratory animals. Ch. 6 in *Evaluation of Novel Protein Products*, A.E. Bender, R. Kihlberg, B. Lofquist and L. Munck (Ed.), p. 117-125. Pergamon Press, New York, New York.

Egum, B.O. 1985. Digestibility of plant proteins: Animal studies. Ch. 11 in *Digestibility and Amino Acid Availability in Cereals and Oilseeds*, J.W. Finely and D.T. Hopkins (Ed.), p. 275-284. American Association of Cereal Chemists, Inc., St. Paul, Minnesota.

Eliason, M. 1996. Utilization of barley. http://www.agric.gov.ab.ca/crops/barley/util02.html (accessed on September 16, 2002).

FAO. 2001. FAO statistical data base. At http://fao.org. Agriculture: Food supply: Crops primary equivalent, livestock and fish primary equivalent (accessed on September 18, 2003).

FAO. 2002. FAO statistical data base. At http://fao.org. Agriculture: Agricultural production: Crops primary, livestock processed (accessed on September 18, 2003).

Garcia, M.C., Torre, M., Marina, M.L., and Laborda, F. 1997. Composition and characterization of soybean and related products. Crit. Rev. Food. Sci. Nutr. 37(4): 361-391.

Glicksman, M. 1986. Functional properties of hydrocolloids. Ch. 3 in *Food Hydrocolloids*, IRL Press (Ed.), p. 47-99. Washington, D.C.

Godon, B., and Willm, C., (Eds). 1994. *Primary Cereal Processing: A Comphresive Source book.* VCH Publishers, Inc., New York, New York.

Health Canada. 1997. Discussion Document: Functional foods and nutraceuticals. Consultation Workshop, Novotel, Ottawa, Ontario.

Helm, J.H. 1992. What is feed quality? Twenty-five years of questions. Barley Newsletter. 36: 189-189.

Henkel, J. 2000. FDA Consumer magazine. Soy: Health claims for soy protein, questions about other components. At http://www.fda.gov/fdac/features/2000/300_20y.html (accessed on October 3, 2003).

Hermansson, A.M. 1978. Phsico-chemical aspects of soy proteins structure formation. J. Texture. Stud. 9: 33-58.

Hettiarachchy, N.S., Griffin, V.K., and Gnanasambandan, R. 1996. Preparation and functional properties of a protein isolate from defatted wheat germ. Cereal Chem. 73(3): 363-367.

Hickling, D.R. 1993. Feed Grains—Utilization and Processing. Ch. D-13 in *Cereals and Oilseeds: Handling, Marketing, Processing*, Canadian International Grains Institute (Ed.), p. 991-1042. Canadian international Grains Institute, Winnipeg, Manitoba.

Hoseney, C.R. (Ed.). 1994. *Principles of Cereal Science and Technology*, 2nd edition. American Association of Cereal Chemists, Inc., St. Paul, Minnesota.

Hudson, C.A., Chiu, M.M., and Knuckles, B.E. 1992. Development and characteristics of high-fibre muffins with oat bran, rice bran, or barley fibre fractions. Cereal Foods World. 37(5): 373-378.

Ingversen, J., and Koie, B. 1973. Lysine rich proteins in the salt-soluble protein fraction of barley. Phytochemistry. 12: 73-98.

Jadhav, S.J., Lutz, S.E., Ghorpade, V.M., and Salunkhe, D.K. 1998. Barley: Chemistry and value-added processing. Crit. Rev. Food Sci. 38(2): 123-171.

Jeknins, A.L., Jenkins, D.J.L., Zdravkovic, U., Wursch, P., and Vuksan, V. 2002. Depression of the glycemic index by high levels of β-glucan fibre in two functional foods tested in type 2 diabetes. European Journal of Clinical Nutrition. 56: 622-628.

Kahlon, T.S., Chow, F.I., Knuckles, B.E., and Chiu, M.M. 1993. Cholesterol-lowering effects in hamsters of β-glucan-enriched barley fraction, dehulled whole barley, rice bran, and oat bran and their combinations. Cereal Chem. 70: 435-440.

Kent, N.L., (Ed.). 1975. *Technology Of Cereals*, 2nd edition. Pergamon of Canada Ltd., Toronto, Canada.

Kinsella, J.E. 1976. Functional properties of proteins in foods: A survey. Crit. Rev. Food. Sci. Nutr. 16(5): 219-280.

Kinsella, J.E. 1981. Relationships between structure and functional properties of food proteins. Ch. 3 in *Food Proteins*, P.F. Fox and J.J. Condon (Ed.), p. 51-104. Applied Science Publishers, New York, New York.

Kirkman, M.A., Shewry, P.R., and Miflin, B.J. 1982. The effect of nitrogen nutrition on the lysine content and protein composition of barley seeds. J. Sci. Food Agric. 33: 115-127.

Klopfenstein, C.F. 1988. The role of cereal beta-glucans in nutrition and health. Cereal Foods World. 33(10): 865-868.

Koie, B., and Nielsen, G. 1977. Extraction and separation of hordeins. Ch. 2 in *Technicques for the Separation of Barley and Maize Seed Proteins*, B. J. Miflin and

P.R. Shewry (Ed.), p. 25-35. Commission of the European Communities, Luxembourg, Belgium.

Koszewski, W., and Jones, G. 2002. Nebraska cooperative extension NF02-546. Soy health claims. At http://www.ianr.inl.edu/pubs/foods/nf546.html.

Landry, J., Moureaux, T., and Heut, J.C. 1972. Extractability of barley seed proteins. Bios (Paris). 3(7-8): 281-292.

Lasztity, R. 1984. Barley proteins. Ch. 5 in *The Chemistry of Cereal Proteins*, R. Lasztity (Ed.), p. 117-130. CRC Press, Inc., Boca Raton, Florida.

Linko, R., Lapvetelainen, A., Laakso, P., and Kallio, H. 1989. Protein composition of a high-protein barley flour and barley grain. Cereal Chem. 66(6): 478-482.

Liu, K., (Ed.). 1999. *Soybeans: Chemistry, Technology and Utilization*. Aspen Publishers, Inc., New York, New York.

MacGregor, A.W., and Fincher, G.B. 1993. Carbohydrates of the barley grain. Ch. 3 in *Barley: Chemistry and Technology*, A.W. MacGregor and R.S. Bhatty (Ed.), p. 73-130. The American Association of Cereal Chemists, Inc., St. Paul, Minnesota.

Mac Key, J. 1981. Cereal production. Ch. 1 in *Cereals: A Renewable Resource, Therory and Practice*, Y. Pomeranz and L. Munck (Ed.), p. 5-24. The American Association of Cereal Chemists, Inc., St. Paul, Minnesota.

Mahan, K.L., and Escott-Stump, S. (Ed.). 2000. *Krause's Food, Nutrition, & Diet Therapy*, 10th ed. W.B. Saunders Company., Toronto, Ontario.

Matz, S.A. (Ed.). 1991. *The Chemistry and Technology of Cereals as Food and Feed*, 2nd edition. Van Nostrand Reinhold., New York, New York.

Mclelland, M. 2002. Current barley production. http://www.agric.gov.ab.ca/crops/stats01.html (accessed on November 13, 2002).

McMurry, J. 1996. Biomolecules: Amino acids, peptides, and proteins. Ch. 27 in *Organic Chemistry: 4th edition*, J. McMurry (Ed.), p. 1055-1098. Brooks/Cole Publishing Campany, Toronto, Canada.

Miflin, B.J., and Shewry, P.R., (Eds.). 1977. *Techniques for the separation of barley and maize proteins*. Commission of the European Communities, Luxembourg.

Morrison, W.R., Scott, D.C., and Karkalas, J. 1986. Variation in the composition and physical properties of barley starches. Starch/Starke. 38: 374-379.

Munck, L. 1981. Barley for food, feed and industry. Ch. 21 in *Cereals: A Renewable Resource, Therory and Practice*, Y. Pomeranz and L. Munck (Ed.), p. 427-461. The American Association of Cereal Chemists, Inc., St. Paul, Minnesota.

Myllarinen, P., Autio, K., Schulman, A.H., and Poutanen, K. 1998. Heat-induced structural changes of small and large barley starch granules. J. Inst. Brew. 104: 343-349.

Nakai, S., Ho, L., Helbig, N., Kato, A., and Tung, M.A. 1980. Relationship between hydrophobicity and emulsifying properties of some plant proteins. Can. Inst. Food Sci. Technol. J. 13(1): 23-27.

Nakai, S. 1983. Structure-function relationships of food proteins with an emphasis on the importance of protein hydrophobicity. J. Agric. Food Chem. 31(4): 677-683.

Newman, C.W., and Newman, R.K. 1989. Nutritional quality of barley for livestock and poultry feed. Barley Newsletter. 33: 142-142.

Newman, R.K., Newman, W.C., and Graham, H. 1989. The hypocholesterolemic function of barley β-glucans. Cereal Foods World. 34(10): 883-885.

Newman, R.K., and Newman C.W. 1991. Barley as a food grain. Cereal Foods World. 36(9): 800-805.

Nierle, W. 1985. Views on the amino acid composition of grain and the influenceof processing. Ch. 3-3 in *Amino Acid Composition and Biological Value of Cereal Proteins*, R. Lasztity and M. Hidvegi (Ed.), p. 371-382. D. Reidel Publishing Company., Dordrecht, Holland.

Phillips, L.G., Whitehead, D.M., and Kinsella, J. (Ed.). 1994. *Structure-Function Properties of Food Proteins*. Academic Press, Inc., Toronto, Ontario.

Pomeranz, Y. 1971. Principles of milling. Ch. 4 in *Wheat: Chemistry and Technology*, Y. Pomeranz (Ed.), p. 115-171. American Association of Cereal Chemists., St. Paul, Minnesota.

Pomeranz, Y. 1973. Industrial uses of barley. Ch. 11 in *Industrial Uses of Cereals*, Y. Pomeranz (Ed.), p. 371-392. American Association of Cereal Chemists., St. Louis, Missouri.

Pomeranz, Y. 1974. Food uses of barley. Crit. Rev. Food Technol. January: 377-394

Pomeranz, Y. 1987. Composition. Ch. 4 in *Modern Cereal Science and Technology*, Y. Pomeranz (Ed.), p. 40-53. VCH Publishers, Inc., New York, New York.

Pomeranz, Y. (Ed.). 1991. *Functional Properties of Food Components*, 2nd ed. Academic Press, Inc., Toronto, Ontario.

Qureshi, A.A., Burger, W.C., Peterson, D.M., and Elson, C.E. The structure of an inhibitor of cholesterol biosynthesis isolated from barley. J. Biol. Chem. 261: 1054-1058.

Rhodes, A.P., and Gill, A.A. 1980. Fractionation and amino acid analysis of the salt-soluble protein fractions of normal and high-lysine barleys. J. Sci. Food Agric. 31: 467-473.

Shewry, P.R., Pratt, H.M., Charlton, M.J., and Miflin, B.J. 1977. Two-dimensional separation of the prolamins of normal and high lysine barley (*Hordeum vulgare* L.). J. Exp. Bot. 28(104): 597-606.

Shewry, P.R., Hill, J.M., Pratt, H.M., Leggatt, M.M., and Miflin, B.J. 1978. An evaluation of techniques for the extraction of hordein and glutelin from barley seed and a comparison of the protein composition of Bomi and Riso 1508. J. Exp. Bot. 29(110): 677-692.

Shewry, P.R., and Kreis, M. 1988. The development and composition of barley grain: Relationship to end use, and potential for manipulation. In *Alternative End Uses of Barley*, D.H.B. Sparrow, R.C.M. Lance and R.J. Henry (Ed.), p. 61-66. Cereal Chemistry Division of the Royal Australian Chemical Institute., Waite, Australia.

Shewry, P.R., Halford, N.G., and Tatham, S. 1994. Analysis of wheat proteins that determine breadmaking quality. Food. Sci Technol. Today. 8(1): 31-36.

Singh, U., and Sastry, V.S. 1977. Studies on the proteins of the mutants of barley grain: I. Extraction and electrophoretic characterization. Cereal Chem. 54(1): 1-12.

Smith, A.K., and Circle, S.J., (Eds.). 1978. Soybeans: Chemistry and Technology. AVI Publishing Co., Westport, CT.

Statistics Canada. 2001. Field crop series. 80(8). Statistics Canada., Ottawa, Ontario.

Swanson, R.B., and Penfield, M.P., 1988. Barley flour level and salt level selection for a whole-grain bread formula. J. Food Sci Off Publ Inst Food Technol. 53(3): 896-901.

Temelli, F. 1997. Extraction and functional properties of barley β-glucan as affected by temperature and pH. J. Food Sci. 62(6): 1194-1197, 1201.

Toufeili, I., Habbal, Y., Shadarevian, S., and Olabi, A. 1999. Substitution of wheat starch with non-wheat starches and cross-linked waxy barley starch affects sensory properties and staling of Arabic bread. J Sci Food Agric. 79: 1855-1860.

Vanicek, F., and Turek, B. 1985. Amino acid problem of cereals at daily meals. Ch. 1-4 in *Amino Acid Composition and Biological Value of Cereal Proteins*, R. Lasztity and M. Hidvegi (Ed.), p. 57-66. D. Reidel Publishing Company., Dordrecht, Holland.

Vasanthan, T., and Bhatty, R.S. 1996. Physicochemical properties of small- and large-granule starches of waxy, regular, and high-amylose barleys. Cereal Chem. 73(2): 199-207.

Wall, J.S. 1979. Properties of proteins contributing to functionality of cereal foods. Cereal Foods World. 24(7): 288-313.

Wilson, C.M., Shewry, P.R., Faulks, A.J., and Miflin, B.J. 1981. The extraction and separation of barley glutelins and their relationship to other endosperm proteins. J. Exp. Bot. 32(131); 1287-1293.

Wolf, M.J., Khov, V., and Seckinger, H.L. 1967. Subcellular structure of endosperm protein in high-lysine and normal corn. Science. 157, 556.

Wolf, W.J. 1970. Soybean proteins: Their chemical and functional properties. J. Agric Food Chem. 18: 969-976

Wolf, W.J., and Cowan, J.C., (Eds.). 1975. *Soybean as a Food Source*. CRC Press, Cleveland, OH.

Wolf, W.J. 1981. Progress and future needs for research in soya protein utilization and nutrition. J. Am. Oil Chem. Soc. 58: 467-473.

Wood, P.J. 1984. Physiological properties and technological and nutritional significance of cereal β-glucans. Ch 1 in *Cereal Polysaccharides in Technology and Nutrition*, V. Rasper (Ed.), p. 35-77. The American Association of Cereal Chemists, Inc., St. Paul, Minnesota.

Woodward, J.R., Ficher, G.B., and Stone, B.A. 1983. Water soluble (1-3), (1-4)-β-D-glucans from barley (Hordeum vulgare) endosperm. II. Fine structure. Carbohydr. Polym. 3: 207-225.

Wu, Y.V., Cluskey, J.E., Wall, J.S., and Inglett, G.E. 1973. Oat protein concentrates from a wet-milling process: Composition and proterties. Cereal Chem. 50: 481-488.

Wu. Y.V., and Sexon, K.R. 1975. Preperation of protein concentrate from normal and high-protein wheats. J. Agric. Food Chem. 23(5): 903-905.

Wu. Y.V., and Sexon, K.R. 1976a. Protein concentrate from normal and high-lysine corns by alkaline extraction: Preparation. J. Food Sci. 41: 509-511.

Wu. Y.V., and Sexon, K.R. 1976b. Protein concentrate from normal and high-lysine corns by alkaline extraction: Composition and properties. J. Food Sci. 41: 512-514.

Wu, Y.V., Sexon, K.R., and Wall, J.S. 1976. Triticale protein concentrate: Preparation, composition and properties. J. Agric. Food Chem. 24(3): 511-517.

Wu. Y.V. 1978. Protein concentrate from normal and high-lysine sorghums: Preperation, composition, and properties. J. Agric. Food Chem. 26(2): 305-308.

Wu, Y.V., Sexon, K.R., and Sanderson, J.E. 1979. Barley protein concentrate from high-protein, high-lysine varieties. J. Food. Sci. 44: 1580-1583.

Yamamoto, S., Sobue, T., Kobayashi, M., sasaki, S., and Tsugane, S. 2003. Soy, isoflavones, and breast cancer risk in Japan. J. Natl. Cancer Inst. 95(12): 906-913

Zayas, J.F. (Ed.). 1997. *Functionality of Proteins in Food.* Springer, Berlin, Germany.

3. QUANTIFICATION AND DISTRIBUTION OF PROTEINS IN BARLEY PEARLING FRACTIONS[1]

3.1. INTRODUCTION

Fractionation of barley grains into its various components may be an effective way to isolate and concentrate its chemical constituents. Extraction parameters can be manipulated to obtain fractions varying in physical, chemical and functional characteristics. A typical barley grain consists of three main tissues; bran, endosperm and embryo (germ). The bran and germ contain majority of the water- and salt-soluble proteins (i.e. albumins and globulins) as well as lipids and minerals. Other proteinaceous materials, such as enzymes that degrade polysaccharides (amylases, gluconases) and proteins (proteases and peptidases) and enzyme inhibitors are present throughout the grain in various tissues (Lasztity 1984). Starch and β-glucan are mostly present in the endosperm along with the storage proteins, consisting of hordeins and glutelins.

The utilization of barley as a human food source is undermined due to the abundance of wheat, rice and corn. Of the total Canadian barley production, 10% is used for malt, 85% is used for animal feed and approximately 5% is used for human food purposes (Bhatty 1986). But the human consumption of barley is slowly increasing mainly due to the health awareness of both soluble and insoluble dietary fibre. Barley β-glucan, which is a major soluble fibre component, is located in the cell walls of the endosperm and is associated with lowering serum cholesterol and possessing antiglycemic properties in both animals and human subjects (Fastnaught et al 1996). On average, barley contains 2-

---

[1] A version of this chapter is to be submitted to the Journal of Agricultural and Food Chemistry for consideration for publication.

11% β-glucan (MacGregor and Fincher 1993). In addition to β-glucan, whole grain barley also contains high concentrations of vitamin E (a fat-soluble vitamin) made up of isomers of tocopherols and tocotrienols. Tocol (tocopherols and tocotrienols) content of barley is in the range of 42-80 mg/kg of grain. The biological functions of tocols include antioxidant activity and reduction of serum LDL-cholesterol in chickens (Peterson 1994; Bhatty 1999). Peterson (1994) reported significantly higher amounts of tocols in the aleurone, subaleurone (bran) and germ tissues of barley as compared to the central region of the endosperm, indicating that tocols are mainly concentrated in the outer layers of the kernel.

Commercially, whole barley grain is exposed to an abrasion process to obtain pot barley, in which 7-14% of the outer kernel is removed or pearl barley where 14-50% of the kernel is abraded (Klamczynski et al 1998). Pot and pearled barley are used in a variety of different foods, ranging from rice extender, casseroles, soups, stews, porridge and baby food (Munck 1981). Furthermore, pot and pearled barley can be milled into flour, which can then be used as a food thickener (due to the high starch and β-glucan contents) or can be substituted for wheat flour in products such as cookies, noodles, muffins, pancakes and extruded cereals (Bhatty 1993). The bran or fines from such commercial processing is considered a byproduct and is used as a supplement in animal feed (cattle and swine).

In North America, traditional protein sources include meat, eggs and milk, whereas in developing parts of the world such as Asia and Africa, diets rich in cereals, oilseeds and lentils are prevalent. Essential amino acid profiles of barley and oat are similar and better than that of wheat, corn and rice (Chung and Pomeranz 1985). However, wheat is the major grain cereal of choice in baked goods due to the functional properties (cohesiveness and elasticity) of wheat gluten. In terms of nutrient content, barley proteins (as well as proteins of other cereals) are first limiting in lysine and second limiting in methionine using the egg-reference pattern (Pomeranz 1974). To elevate its lysine content, attempts have been made to cultivate high-protein barley by increasing nitrogen fertilization. Kirkman et al (1982) showed that even though the total protein increased in the whole barley grain, the relative lysine concentration was negatively correlated with the increase in grain nitrogen. The extra nitrogen in the seed was utilized in the synthesis of hordein storage proteins in the endosperm, which are high in glutamic acid and proline and very low in lysine.

High-lysine barley varieties such as Hiproly, Emir and Riso have also been developed. These varieties have a much higher essential amino acid complement as compared to other grains including normal barley, oat and mutant corn (Pomeranz 1974). Much of the lysine content (80-90%) is present mainly in albumins and globulins of the aleurone and glutelins in the endosperm (Ingversen and Koie 1973; El-Negoumy 1977). The drawback of high-lysine barleys in terms of commercial cultivation is their poor agronomical characteristics such as lower grain yield and shrunken kernels (Pomeranz 1974). Low concentration of hordeins in high-lysine barley disrupts normal grain metabolism by impairing the synthesis of other seed components (i.e. carbohydrates) resulting in low seed weight and decreased functionality (Kirkman et al 1982).

Pearling is an abrasive process that gradually removes the seed coat (testa and pericarp), aleurone, subaleurone and germ leaving behind a polished grain. It is an effective process of dry fractionating cereal components and concentrating them in specific isolated tissues. Zheng et al (2000) collected pearled fines at 10% intervals by successive abrading. Protein content was highest in the 10-30% pearl fraction, whereas fractions 50-70% were higher in starch with β-glucan being concentrated in the 50-60% pearling fraction. Bhatty (1992, 1995) dry milled barley and oat samples to obtain approximately 30% bran in which β-glucan content increased by approximately 37-38% as compared to the whole grain.

Robbins and Pomeranz (1972) hand dissected the hull (lemma and palea) and germ and evaluated their composition. Barley hulls, which represent 10% of the grain, were very low in protein (<2%) while the germ (~4% of the grain) was very high in protein (35%). Pearling fines of 20% had protein content and amino acid values intermediate to 8% and 53% pearling fractions (Robbins and Pomeranz 1972). Sumner et al (1985) used a dehuller to obtain fines fractions corresponding to 17% pearlings, which were higher in protein, oil and amino acid scores as compared to the whole grain. Bhatty (1997) showed that bran (pericarp, testa, aleurone, subaleurone and germ) constituted approximately 30% (w/w) of the whole barley grain. Bran from hull-less barley varieties were either obtained by using a Satake mill (rice pearler) or a Buhler roller mill. Bran fractions (34%) were high in starch and contained 34% more β-glucan, but the protein content was higher in the pearled grain fraction and lower in the bran as compared to the whole grain.

There is limited data in terms of pearling studies to maximize the distribution of barley components in two basic flour fractions, mainly the endosperm and bran. Gohl et al (1977) did sequential abrasion of barley where 10-24% pearling fractions contained high protein and fat with minimal starch contamination; however, β-glucan content of the fractions was not determined and chemical analysis of the pearled grain was not done. Wu et al (1994) have isolated enriched protein and β-glucan fractions by either sieving or air classification. They obtained flour fractions differing in particle size and yield. Grain variety had a significant effect on the results, where protein content in normal barley was greatest after the air classification process (no sieving), whereas for high protein and high β-glucan barley variety, β-glucan content was significantly higher after sieving (no air classification).

Barley, similar to other cereals, contain all four classes of proteins (albumins, globulins, hordeins and glutelins). Pearling of barley grain could be a potentially useful technique in isolating protein classes, thus concentrating the essential amino acids in the bran. As compared to the whole grain, pearling flour (bran) of regular barley was found to be higher in protein content and lower in hordeins and the amino acid profile was similar to that of high-lysine varieties (Hofman 1975). The pearling process can be manipulated to obtain fractions of optimal compositional properties, which can then be used in specific end products. Many of the pearling studies using oats and barley have concentrated on the extraction of β-glucan and not much attention has been given to the distribution of proteins. Therefore, the objectives of the study were:

1. to determine the effects of pearling level on the distribution of protein, β-glucan and starch in the pearling fractions obtained,
2. to extract and quantify albumins, globulins, prolamins and glutelins in the whole grain, pearling flour and pearled grain flour of optimal pearling fractions, and
3. to determine the amino acid profile in the whole grain and optimal pearling fractions as well as in their isolated protein classes.

3.2. MATERIALS AND METHODS

3.2.1. Materials

CDC Candle, a hull-less, waxy barley variety was provided by Agricore United, Calgary, AB. Condor barley (breeders seed), which is a hull-less, regular cultivar was provided by the Field Crop Development Center, Alberta Agriculture, Food and Rural Development (AAFRD), Lacombe, AB.

3.2.2. Pearling

All pearling work was done at the Center for Agri-Industrial Technology (AAFRD), Edmonton, AB. For both barley varieties (25 kg samples), the grains were cleaned of debris using a Duo-aspirator (Style COS3, Carter Day International, Inc., Minneapolis, MN) and sized using a Crippen micro 180 grain sizer (Model DCX 202E, Crippen Manufacturing Co., St. Louis, MO) equipped with 2.73, 2.34 and 2.08 mm screens. Grains within the size range of 2.34-2.73 mm was used in the subsequent pearling step. Barley grain samples (1-1.5 kg) were pearled 5-45% using a Westrup dehuller (Model 970218, Westrup Co., Sorovej, Denmark). Constant pearling speed of 3900 rpm was used and time of pearling was varied from 3 to 30 min to achieve the desired pearling level. The combined pearling flour (PF) and pearled grain were sieved using a SWECO vibro-energy separator (Model 6033 E, SWECO Inc., Toronto, ON) equipped with a 1.76 mm screen and the pearled grain was milled into flour using a Ultra Centrifugal mill (Type ZM 100, Retsch, Hann, Germany). Weight of the pearling flour was determined and percentage of pearling was reported as the weight of PF over the total grain weight.

3.2.3 Compositional analysis

β-Glucan and starch determinations were done according to McCleary and Glennie-Holmes (1985) and McCleary et al (1994), respectively, using Megazyme assay kits (Megazyme Inc., Wicklow, Ireland). Ash and moisture contents were determined using AACC method 08-01 and AACC method 44-19, respectively (AACC, 1982). Lipid content was determined according to AACC method 30-25 (AACC, 1982) and protein content (%N x 6.25) was determined using a Nitrogen analyzer (Model FP 428, Leco Corp., St. Joseph, MI). All analyses were carried out in duplicate.

3.2.4. Scanning electron microscopy

For both varieties, pearled grain kernels were randomly selected from whole and pearled grains. Two grains were mounted on semi-circular stubs using double sided carbon sticky tape and colloidal graphite solution was applied to the bottom and sides of the grains for conductivity. The mounted grains were sputtered with 100 Å of gold and examined using a JEOL scanning electron microscope (Model JSM 6301FXV, JOEL, Ltd., Tokyo, Japan) with an acceleration voltage of 5 kV.

3.2.5. Color determination

Duplicate color measurements of whole grain flour (WGF), pearling flour (PF) and pearled grain flour (PGF) of both varieties were done using a LabScan XE Hunterlab color difference meter (Model D52-2, Hunter Associates Laboratory, Fairfax, VA). Color values for Hunter *L*, *a* and *b* parameters were recorded.

3.2.6. Protein fractionation

Albumins, globulins, hordeins and glutelins were extracted from whole grain flour (WGF), 19% pearling flour (19% PF) and 19% pearled grain flour (19% PGF) from both varieties according to modified procedures of Koie and Nielsen (1977) and Shewry et al (1978) as shown in Figure 3.1. Extraction of protein classes was done at room temperature (23°C) using two sequential steps of 1 hr and 0.5 hr and the supernatants were combined. A solvent-to-feed ratio of 10:1 was used and each extraction was done in duplicate.

Albumins and globulins were extracted from 25 g flour samples using 250 mL solution of 0.5*M* NaCl and 0.054*M* sodium ethylenediaminetetraacetic acid (Na-EDTA). The mixture was centrifuged (Model J2-21, Beckman Instrument Co., Mississauga, ON) for 15 min at 18,000 x *g* at 25°C to remove solids. The salt soluble extract contained in the supernatant was dialyzed (5,000 Da, i.e. molecular weight cut-off point of membrane) for 24 hr against distilled water (20-25°C). The precipitated globulins were separated by centrifugation and redissolved in distilled water while the supernatant, which contained the water-soluble albumins, was collected and both fractions were freeze-dried.

The remaining solid residue was subjected to another extraction step using a mixture of 70% (v/v) ethanol and 20% (v/v) 2-mercaptoethanol to recover the prolamins (hordeins). Upon centrifugation, the supernatant was rotary evaporated (45°C) to dryness and the remaining solids were redissolved in distilled water and freeze-dried. The final protein fraction (glutelin) was extracted using 0.013*M* sodium borate ($Na_2B_4O_7 \cdot 10\ H_2O$) solution containing 0.04*M* NaOH and 0.5% (w/v) sodium dodecyl sulfate (SDS). After

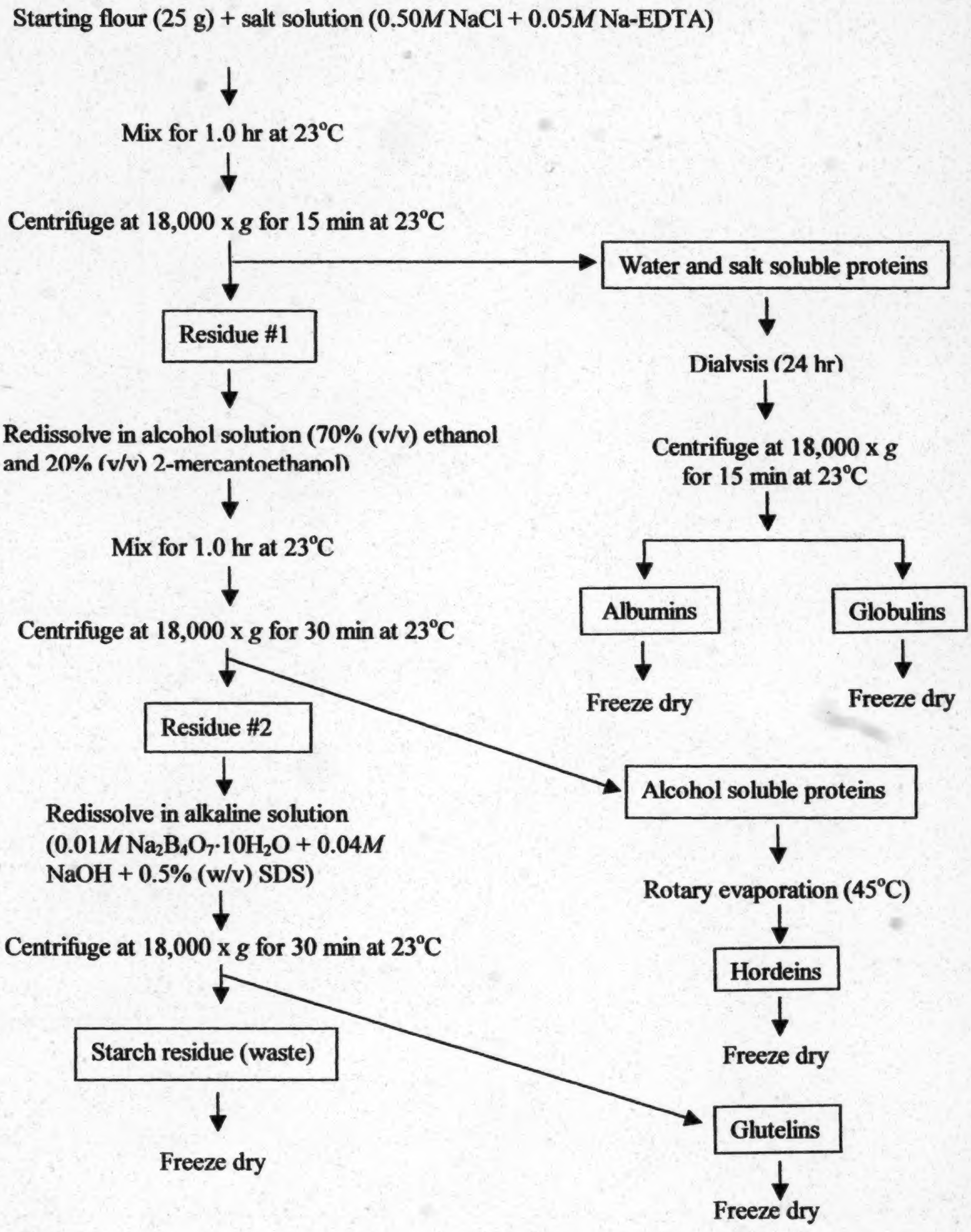
Starting flour (25 g) + salt solution (0.50M NaCl + 0.05M Na-EDTA)
Mix for 1.0 hr at 23°C
Centrifuge at 18,000 x g for 15 min at 23°C
Water and salt soluble proteins
Residue #1
Dialysis (24 hr)
Redissolve in alcohol solution (70% (v/v) ethanol and 20% (v/v) 2-mercaptoethanol)
Centrifuge at 18,000 x g for 15 min at 23°C
Mix for 1.0 hr at 23°C
Albumins
Globulins
Centrifuge at 18,000 x g for 30 min at 23°C
Freeze dry
Freeze dry
Residue #2
Alcohol soluble proteins
Redissolve in alkaline solution (0.01M $Na_2B_4O_7 \cdot 10H_2O$ + 0.04M NaOH + 0.5% (w/v) SDS)
Rotary evaporation (45°C)
Centrifuge at 18,000 x g for 30 min at 23°C
Hordeins
Starch residue (waste)
Freeze dry
Glutelins
Freeze dry
Freeze dry

Figure 3.1. Extraction of protein classes from whole grain barley flour and 19% pearling fractions.

centrifugation, both the supernatant and the remaining residue were frozen and then freeze-dried for analysis.

3.2.7. Amino acid analysis

Isolated protein fractions (albumins, globulins, hordeins and glutelins) from different flour samples (WGF, 19% PF and 19% PGF) were hydrolyzed using AOAC method 994.12 (AOAC 1999) and their amino acid profiles were determined using a modified procedure of Sedgwick et al (1991). Sample weight was adjusted based upon the protein content of samples such that 1 mg protein was analyzed. Cystine and methionine were first oxidized to cysteic acid and methionine sulfone, respectively, using 88% (v/v) performic acid. The remaining amino acids were hydrolyzed using 6*N* HCl and then neutralized with 6*M* NaOH after which internal standards (ethanolamine and β-amino-n-butyric acid) were added. Separation and quantification of amino acids were accomplished using a high performance liquid chromatograph (HPLC) (Model LC506 000-00, Varian Canada, Mississauga, ON) equipped with a fluorichrom detector (Model 2070, Spectrofluorometer, excitation λ 340 nm and emission λ 450 nm) and an auto-injector (Model SIL-9A, Shimadzu Scientific Instruments, Inc., Columbia, MD). Samples (25 μL) were injected into a Supelcosil 3 μm LC-18 reverse phase column (4.6 x 150 mm, Supelco Canada, Oakville, ON) equipped with a guard column (4.6 x 50 mm) packed with Supelco LC-18 reverse phase packing (20-40 μm). Chromatographic peaks were recorded and integrated using a Shimadzu Class-VP Chromatography Data System. Amino acids in the samples were quantified using a amino acid standard solution (Stock No. AA-S-18, Sigma Inc., St. Louis, MO). It was not possible to determine tryptophan and proline contents using this method.

3.2.8. Statistical analysis

Pearling and fractionation of protein classes were done in duplicate. Chemical composition and amino acid determinations of each fraction were also done in duplicate, thus means of four values are reported. Analysis of variance of results was done using General Linear Model (GLM) procedure of SAS Statistical Software, Version 8 (SAS Institute, 1999). The model consisted of the main effects of percentage pearling and flour type and their interactions on starch, protein β-glucan, lipid and ash content. Multiple comparison of means was performed by Tukey's studentized range (HSD) test at α=0.05 level.

3.3. RESULTS AND DISCUSSION

Candle and Condor barley were chosen for this study as the varieties to represent waxy and regular type cultivars. Thus, they substantially differ in their structure and composition. For example, Candle barley is high in β-glucan content, whereas Condor barley is high in protein content. In addition, Candle barley starch (waxy type) has higher amylopectin and lower amylose as compared to Condor barley starch (regular type).

3.3.1. Compositional analysis of barley pearling fractions

For both flour varieties and their pearling fractions (PF and PGF), the moisture content ranged from 10-13%. The compositional analysis results for Candle and Condor barley pearlings are presented in Tables 3.1 and 3.2, respectively. Condor WGF had higher protein (18.1% vs. 14.4%) but lower β-glucan content (6.4% vs. 7.5%) as compared to Candle. Starch content was approximately 58% in WGF of both barley varieties and the lipid content in Candle and Condor WGF was in the range of 2.2-2.6%.

2

Starch concentration of Candle pearlings increased gradually with the pearling level until a constant value of 71-73% was reached at 18-22% PGF (Table 3.1). In the 18-22% pearling flour, the starch content was consistently between 19-21%, but increased sharply at pearling levels of 30% and 39%. Protein content of 8-39% PGF was between 10-14% whereas that of PF was higher, with maximum (28.7% protein) value at 8% pearling and minimum value (21.7% protein) occurring at 39% pearling level. Similar to starch, protein content stabilized between 23-25% in 14-22% PF before decreasing again at higher pearling levels. There was no consistent trend in β-glucan content of Candle barley with an increase in pearling level. There was no significant difference ($P > 0.05$) in the β-glucan content (7.7-8.8%) of PGF up to 22% pearling, but it decreased significantly at higher pearling levels. β-Glucan content of PF increased significantly ($P \leq 0.05$) with pearling level, reaching its highest level of 5.8% at 39% pearling. The lipid content of Candle barley was between 8-11% in 8-22% PF and decreased significantly ($P \leq 0.05$) to 6.5% in 39% PF. Thus, for Candle, maximization of proteins and lipids occurs in 19-22% PF and maximization of starch and β-glucan occurs in 19-22% PGF.

The trend in starch content for PF and PGF of Condor was similar to that of Candle. Starch content reached a consistent value ($P > 0.05$) of 69-71% in 14.5-21% PGF and increased to 73-74% in 30-42% PGF (Table 3.2). Starch content in the 17-19% PF was similar ($P > 0.05$) at 18.4-18.5% and increased ($P \leq 0.05$) to 31% in 42% PF. Protein contents of Condor fractions were more stable as compared to that of Candle fractions. In 5-21% PF, the protein concentration remained between 28-29% and decreased ($P \leq 0.05$) to 26% in 42% PF. β-Glucan content was highest (7.0-7.6%) in 17-21% PGF and in terms of the pearling flour, β-glucan reached a maximum value of 6.2% in 42% PF. The lipid

Table 3.1. Proximate analysis of Candle barley pearling fractions.

Pearling (%)	**Flour fraction**	**% (w/w, dry matter basis)**				
		Starch	**Protein**	**β-glucan**	**Lipid**	**Ash**
0.0	**WGF**	**58.1**	**14.4**	**7.5**	**2.6**	**1.6**
8.0	PF	10.2^{i}	28.7^{a}	2.6^{k}	10.6^{a}	5.8^{d}
	PGF	65.5^{c}	13.7^{h}	8.5^{ab}	2.2^{g}	1.8^{h}
9.0	PF	12.0^{i}	26.7^{b}	3.2^{j}	10.5^{a}	6.1^{a}
	PGF	66.9^{c}	13.4^{i}	8.8^{a}	1.9^{ghi}	1.7^{i}
12.7	PF	14.9^{h}	25.6^{c}	3.7^{i}	9.8^{b}	6.0^{b}
	PGF	70.5^{b}	12.8^{j}	7.7^{abc}	1.6^{hi}	1.5^{j}
14.4	PF	18.0^{g}	24.7^{d}	4.1^{h}	9.2^{cd}	5.8^{c}
	PGF	70.2^{b}	12.7^{jk}	7.8^{abc}	1.6^{hi}	1.4^{k}
18.2	PF	19.2^{fg}	24.6^{d}	4.5^{g}	9.4b^{c}	5.8^{cd}
	PGF	71.0^{b}	12.5^{k}	7.9^{abc}	1.7^{ghi}	1.3^{kl}
19.0	PF	18.6^{fg}	24.8^{d}	4.9^{f}	9.6^{bc}	6.0^{ab}
	PGF	71.1^{b}	11.9^{l}	8.3^{ab}	1.3^{i}	1.0^{n}
21.8	PF	21.1^{f}	23.9^{e}	5.2^{e}	8.7^{d}	5.6^{e}
	PGF	73.2^{ab}	11.8^{l}	8.0^{abc}	1.5^{hi}	1.2^{m}
29.8	PF	30.2^{e}	22.6^{f}	4.6^{g}	7.5^{e}	5.0^{f}
	PGF	71.4^{ab}	12.0^{l}	6.9^{c}	1.4^{hi}	1.3^{l}
38.6	PF	36.4^{d}	21.7^{g}	5.8^{d}	6.2^{f}	4.3^{g}
	PGF	74.4^{a}	10.4^{m}	7.6^{bc}	1.2^{i}	1.0^{n}

$^{a-n}$Means with different letters within a column are significantly different ($P \leq 0.05$).

content in 5-21% PF was in the range of 8-10% whereas it was less than 1% in 21-42% PGF. As was the case with Candle, maximization of proteins and lipids occurred in 19-21% PF and maximization of starch and β-glucan occurred in 19-21% PGF of Condor barley.

In Candle barley, β-glucan content increased by 43% in 19-22% PGF whereas in Condor it increased by only 18% compared to that in whole grain. These results indicate that β-glucan is not restricted to the central endosperm but is also located in the aleurone and cell walls of the outer endosperm. Zheng et al (2000) showed that low β-glucan hull-less barley contained high levels of β-glucan in the subaleurone region of the grain

Table 3.2. Proximate analysis of Condor barley pearling fractions.

Pearling (%)	**Flour fraction**	**% (w/w, dry matter basis)**				
		Starch	**Protein**	**β-glucan**	**Lipid**	**Ash**
0.00	**WGF**	**57.6**	**18.1**	**6.4**	**2.2**	**1.8**
5.3	PF	11.3^{i}	29.4^{a}	2.2^{i}	10.1^{a}	5.2^{a}
	PGF	63.2^{e}	17.8^{f}	5.7^{e}	2.1^{h}	1.6^{h}
7.0	PF	14.4^{hi}	29.2^{ab}	2.6^{h}	10.0^{ab}	5.1^{c}
	PGF	66.4^{d}	17.2^{g}	6.4^{cd}	1.8^{I}	1.4^{I}
11.5	PF	16.2^{h}	28.6^{bc}	3.4^{g}	9.7^{b}	5.2^{ab}
	PGF	66.4^{d}	16.8^{gh}	6.8^{bc}	1.6^{j}	1.3^{j}
14.5	PF	23.4^{g}	28.2^{c}	4.3^{f}	9.3^{c}	5.2^{bc}
	PGF	70.5^{c}	16.5^{hi}	6.5^{bcd}	1.4^{k}	1.2^{k}
17.7	PF	18.4^{h}	28.0^{c}	4.3^{f}	8.8^{d}	5.0^{d}
	PGF	69.2^{c}	16.2^{i}	7.6^{a}	1.0^{m}	1.1^{l}
19.0	PF	18.5^{h}	28.8^{bc}	5.7^{e}	8.8^{d}	5.1^{c}
	PGF	69.2^{c}	15.8^{j}	7.0^{abc}	1.2^{l}	1.0^{lm}
21.0	PF	22.5^{g}	28.0^{c}	4.6^{f}	8.1^{e}	4.8^{e}
	PGF	71.4^{bc}	15.7^{j}	7.1^{ab}	0.8^{n}	1.0^{m}
29.8	PF	29.5^{f}	27.2^{d}	5.5^{e}	6.6^{f}	4.1^{f}
	PGF	74.4^{a}	14.1^{k}	6.1^{de}	0.6^{o}	0.7^{n}
42.1	PF	31.0^{f}	26.1^{e}	6.2^{d}	5.4^{g}	3.5^{g}
	PGF	73.2^{ab}	13.0^{l}	7.0^{bc}	0.6^{o}	0.6^{o}

$^{a-o}$Means with different letters within a column are significantly different ($P \leq 0.05$).

whereas in high β-glucan hull-less barley varieties, substantial amounts of β-glucan were present in the central endosperm. For both Candle and Condor, the concentration of β-glucan in the 19-22% PF was high as compared to that in WGF thus further increasing the nutritional value of this pearling fraction.

Variability in mass balance that occurs in the values given in Tables 3.1 and 3.2 maybe due to non-uniform pearling and error in analysis. Sizing of the grains was done prior to pearling to obtain uniform grain size for the purpose of minimizing variability during the timed pearling step. The pearling values given in Table 3.1 and 3.2 represent an average of all the grains pearled for a particular variety.

3.3.2. Scanning electron microscopy

Figures 3.2 and 3.3 show SEM images of the whole grain and grains pearled to various percentages for Candle and Condor, respectively. In both Candle and Condor whole grain, the pericarp, seed coat, aleurone and subaleurone layers can be seen. In Candle, 18-22% pearling abraded most of the bran. For Condor, the aleurone and subaleurone layers were still present at 18% pearling, whereas at 21% pearling it was completely removed with some damage to the outer endosperm cell walls.

Spherical protein bodies were highly concentrated in the aleurone cells but in the endosperm, both protein bodies and a thin protein matrix (fused protein bodies forming a continuous layer of protein mass) were present. For whole grain barley, Bhatty (1997) showed that the aleurone, subaleurone and the outer regions of the endosperm were heavily stained with Amido Black solution due to the presence of protein bodies while in the 40% pearled grain, the endosperm was less stained due to the presence of starch granules. Yupsanis et al (1990) have reported that the aleurone and embryonic cells of barley and oat contain 7S globulins, which are similar to the vicilin-type 7S globulins of legumes that are partially responsible for the texturization properties of soybean proteins. However, the protein matrix found in the barley endosperm is perhaps due to aggregated hordeins, which surround the starch granules and prevent their hydrolysis by amylases during mashing, resulting in decreased malt quality (Millet et al 1991).

3.3.3. Color analysis

Hunter color values of Candle and Condor barley and their pearling fractions are presented in Table 3.3. Between varieties, there was not much difference in *L* (whiteness), *a* (redness) and *b* (yellowness) values of WGF. Within both varieties, as the level of

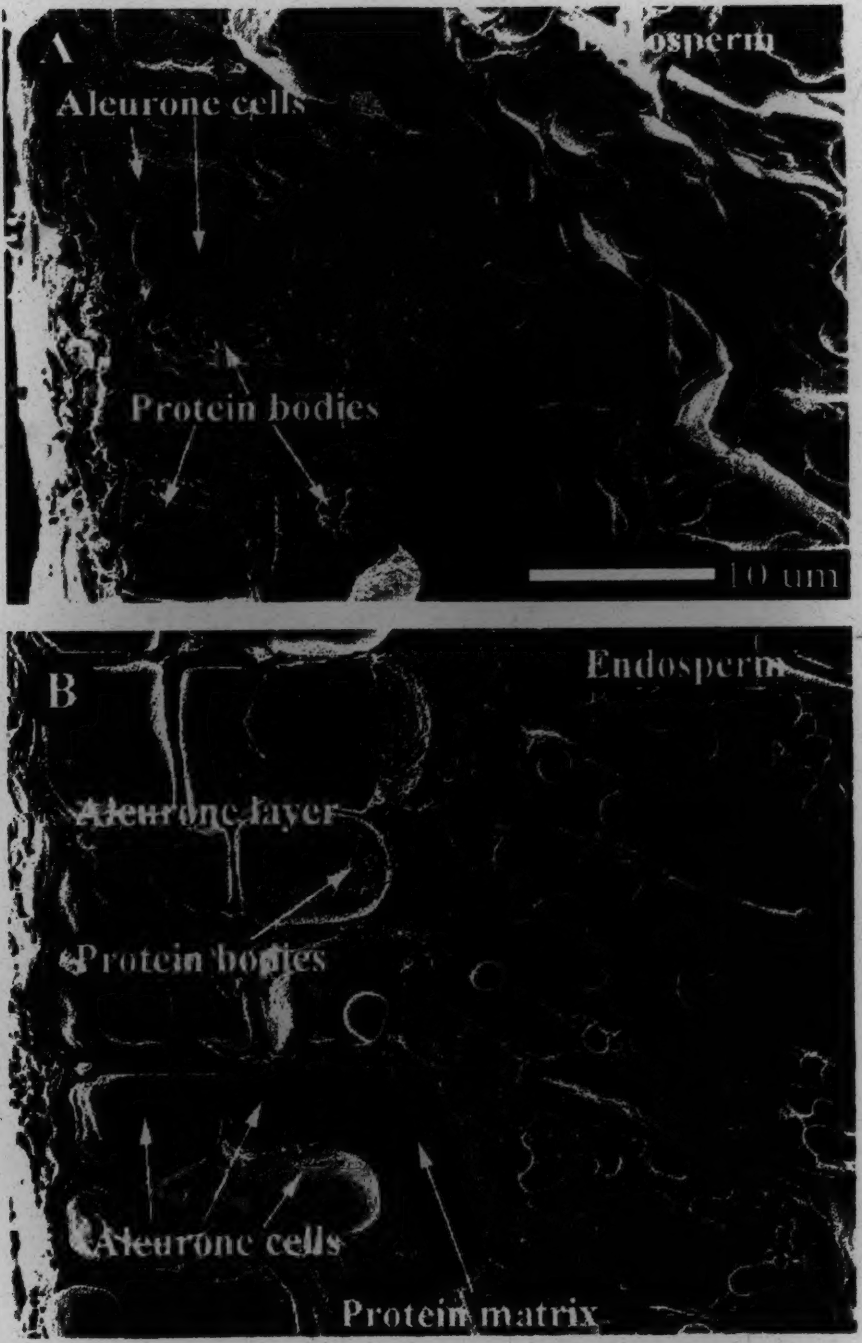
A
Endosperm
Aleurone cells
Protein bodies
10 um
B
Endosperm
Aleurone layer
Protein bodies
Aleurone cells
Protein matrix

Figure 3.2. Scanning electron micrographs of Candle barley whole grain (A), 18% pearling (B), 22% pearling (C), and 39% pearling (D) fractions. Images are at 700x magnification.

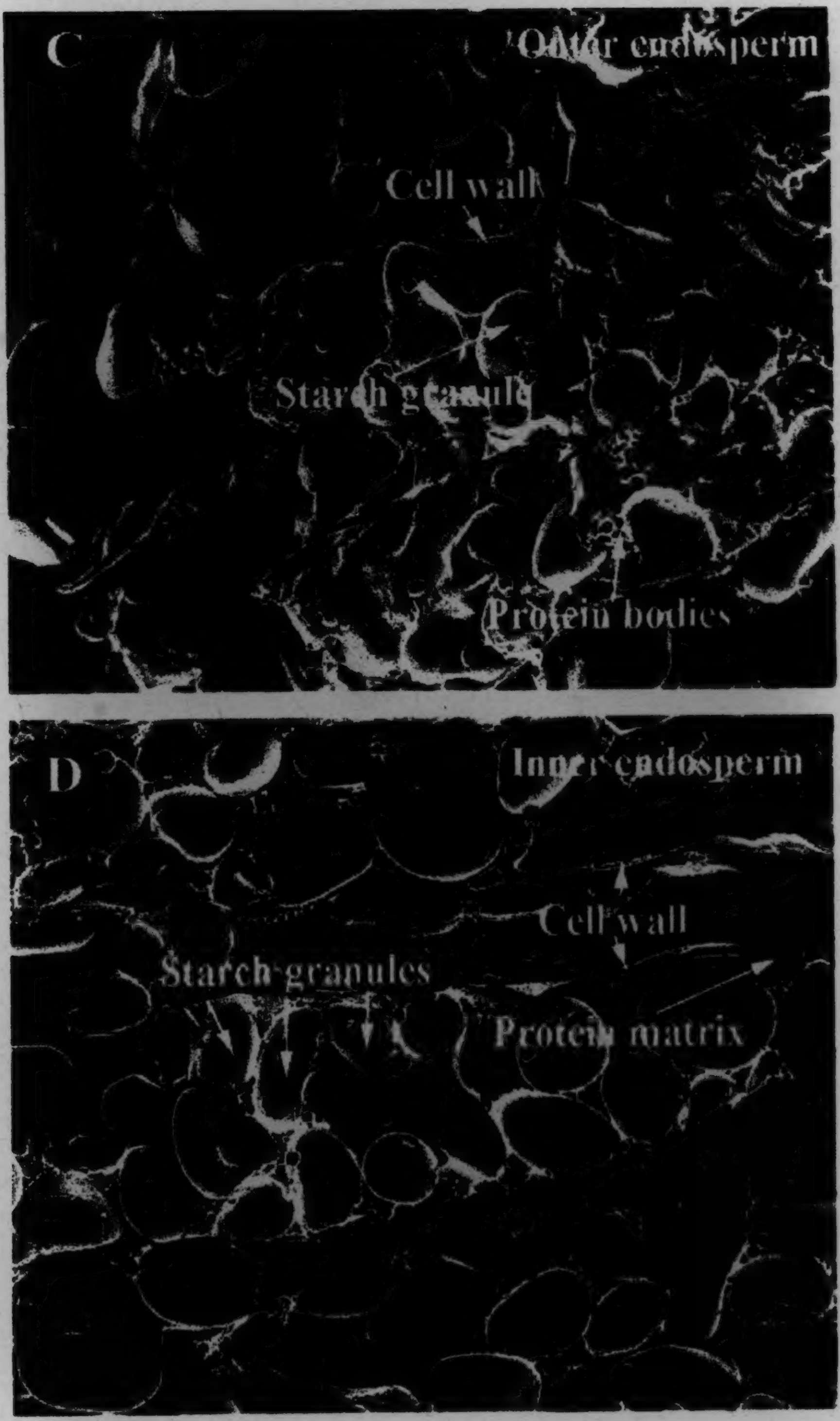
C
Outer endosperm
Cell wall
Starch granule
Protein bodies
D
Inner endosperm
Cell wall
Starch granules
Protein matrix

Figure 3.2. (continued)

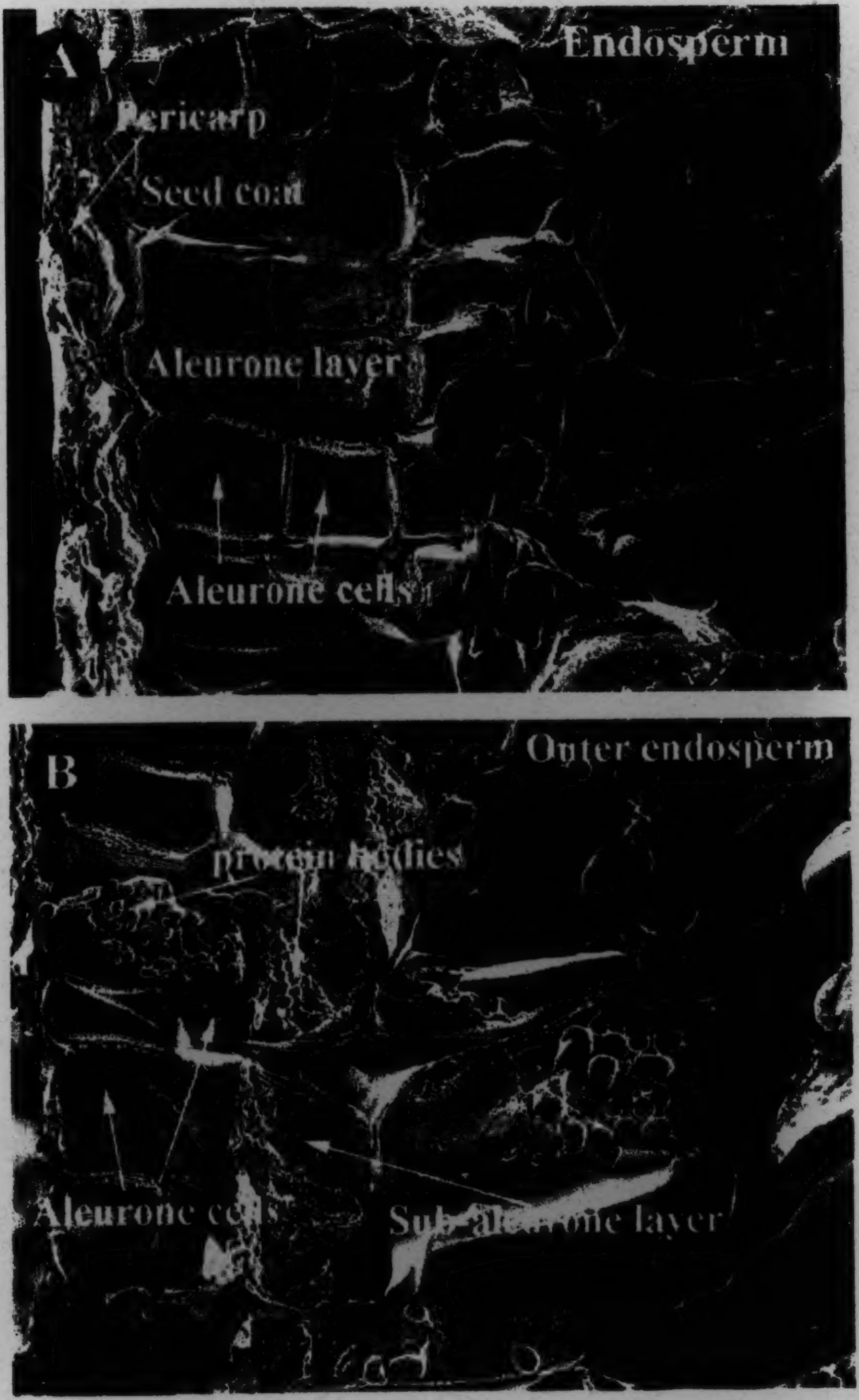
A
Endosperm
Pericarp
Seed coat
Aleurone layer
Aleurone cells
B
Outer endosperm
protein bodies
Aleurone cells
Sub-aleurone layer

Figure 3.3. Scanning electron micrographs of Condor barley whole grain (A), 18% pearling (B), 21% pearling (C), and 42% pearling (D) fractions. Images are at 700x magnification.

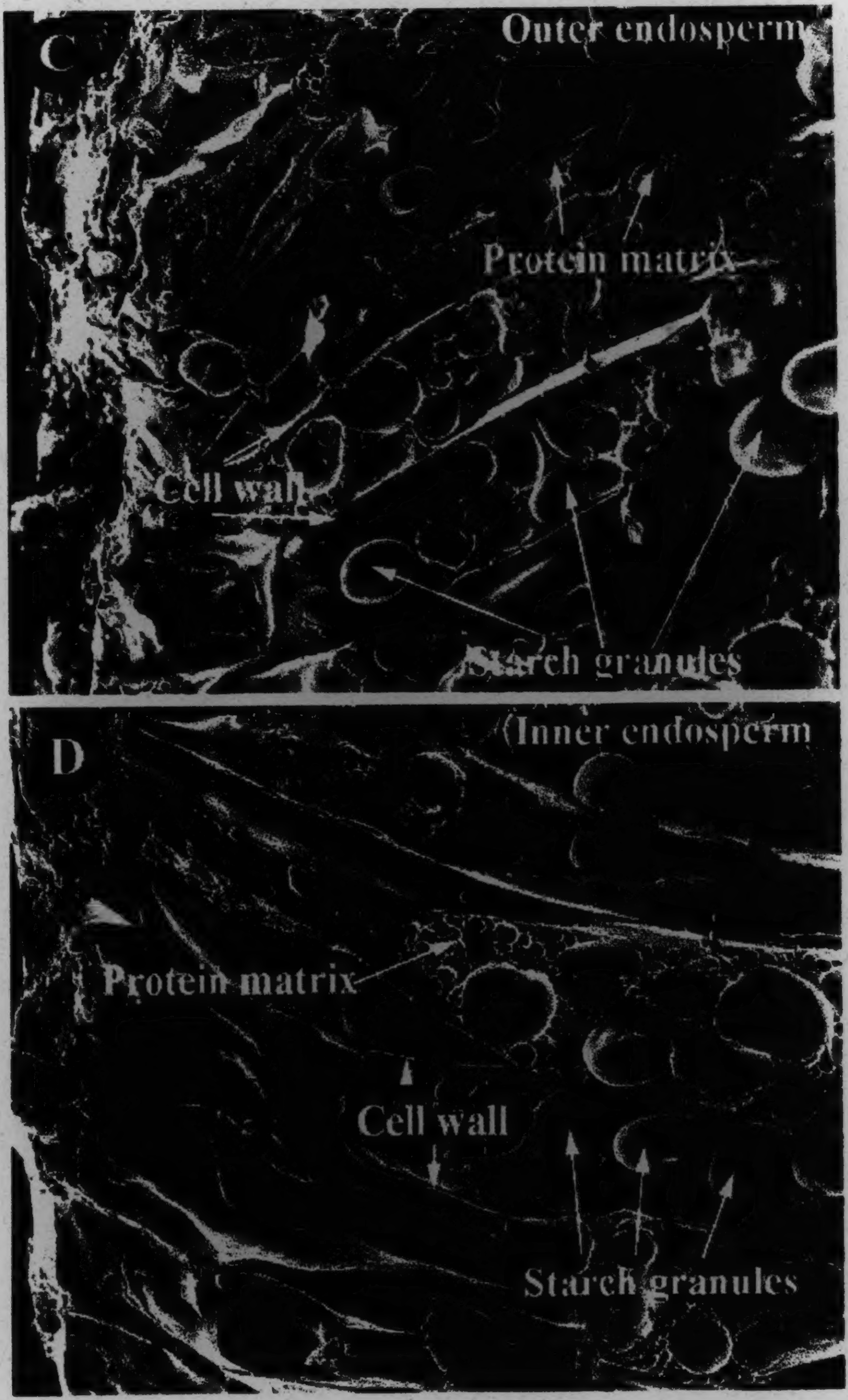
C
Outer endosperm
Protein matrix
Cell wall
Starch granules
D
Inner endosperm
Protein matrix
Cell wall
Starch granules

Figure 3.3. (continued)

Table 3.3. Effect of pearling on the Hunter color values (*L*, *a*, *b*) of Candle and Condor pearling fractions.

Candle					Condor				
Pearling (%)	**Flour fraction**	***L***	***a***	***b***	**Pearling (%)**	**Flour fraction**	***L***	***a***	***b***
0.0	**WGF**	**82.2**	**1.7**	**11.4**	**0.0**	**WGF**	**80.2**	**2.0**	**11.0**
8.0	PF	62.1	5.2	21.8	5.3	PF	61.8	5.1	20.8
	PGF	82.3	1.5	10.5		PGF	81.5	1.7	10.9
9.0	PF	63.5	4.9	21.1	7.0	PF	63.8	4.8	20.4
	PGF	82.8	1.4	10.2		PGF	82.6	1.5	10.2
12.7	PF	66.2	4.4	19.9	11.5	PF	65.2	4.5	20.0
	PGF	84.0	1.1	9.4		PGF	83.2	1.4	9.8
14.4	PF	68.0	4.1	19.3	14.5	PF	66.9	4.2	19.2
	PGF	84.6	1.0	9.1		PGF	83.5	1.4	9.6
18.2	PF	68.8	3.9	18.9	17.7	PF	68.5	4.0	18.7
	PGF	84.9	1.0	8.9		PGF	84.2	1.2	9.0
19.0	PF	72.8	3.5	19.2	19.0	PF	68.4	4.0	18.8
	PGF	85.6	0.9	8.4		PGF	83.7	1.4	9.0
21.8	PF	70.2	3.6	18.2	21.0	PF	69.4	3.8	18.6
	PGF	85.4	0.7	8.4		PGF	84.2	1.2	9.1
29.8	PF	71.6	3.5	17.9	29.8	PF	73.0	3.1	16.8
	PGF	84.9	1.1	8.8		PGF	85.1	1.0	8.0
38.6	PF	73.7	3.0	16.2	42.1	PF	75.3	2.7	15.5
	PGF	86.1	0.8	7.9		PGF	85.4	1.0	7.7

[1] Values are means of two determinations. Standard deviations are <3%. Hunter color values: *L* (0 black, 100 white), *a* ($^-$ green, $^+$ red) and b ($^-$ blue, $^+$ yellow).

pearling increased the *L* value of the PF increased correspondingly with a reduction in both red and yellow color intensities. In Candle, the *L* value stabilized at approximately 85-86 in 18-39% PGF, whereas the *L* value for 19-39% PF reached a level of 70-73. The intensity of yellow color in Candle 8-39% PF gradually decreased from 21 to 16 with increasing pearling level. In Condor, as was the case with Candle, the *L* value stabilized at 83-85 in 19-42% PGF fractions but the *L* value of 5-42% PF increased from 61 to 75. In both barley varieties, yellow color intensity was substantially higher than red color intensity in WGF as well as in PF and PGF fractions. Due to the high ash content of the pearling flour, PF was darkest at lower pearling levels (5-8%) but became lighter due to starch contamination as the level of pearling increased. The red and yellow color of PF is attributed to the presence of anthocyanin pigments in the pericarp and aleurone layer of barley grain (Pomeranz 1987).

3.3.4. Quantification and distribution of protein classes

Quantification of the four major protein classes in WGF, 19% PF and 19% PGF is reported in Table 3.4. Candle WGF had similar albumin and higher globulin contents as compared to Condor, but Condor had substantially higher hordein content with glutelin concentration being similar in both cultivars. Lasztity (1984) and Linko et al (1989) reported an albumin content of 3-5% and glutelin content of 35-45% in high-protein barley flour. Chung and Pomeranz (1985) have summarized the solubility of protein fractions in WGF of various barley cultivars, where the values for albumins ranged from 3 to 24%, globulins were 8-27%, hordeins were 19-59% and glutelins ranged from 12-39% of total grain proteins. In this study, the globulin concentration of 19% PF increased from 8.1 to 12.6% in Candle and 6.0 to 13.0% in Condor with a corresponding decrease in hordein content as compared to WGF. In 19% PGF, concentrations of albumin and globulins were lower but hordein content was higher as compared to WGF and 19% PF. Glutelin contents of WGF, 19% PF and 19% PGF of each barley variety were similar.

Distribution of the protein classes among the pearling fractions is shown in Table 3.5. In Candle, albumins, hordeins and glutelins were concentrated to a higher extent in 19% PGF as compared to 19% PF. In Condor, the same trend was evident with the exception of globulin, which was higher in the 19% PF of Condor whereas it was distributed evenly in Candle. These results indicate that both hordeins and glutelins are concentrated in the central part of the endosperm whereas albumins and globulins may also be present in the peripheral regions of the endosperm, which was not abraded by 19%

Table 3.4. Quantification of the four major protein classes in whole grain and 19% pearling fractions of Candle and Condor Barley.

Variety/Flour	Protein classes (% w/w of total proteins)[1]				
	Albumin	**Globulin**	**Hordein**	**Glutelin**	**Residue**
Candle/WGF	12.3±0.4	8.1±0.2	35.5±4.2	21.6±0.6	7.6±0.4
19% PF	13.4±0.1	12.6±0.0	27.5±1.1	22.0±0.2	9.8±0.2
19% PGF	9.6±0.5	6.3±0.1	42.5±1.0	23.2±0.2	1.6±0.4
Condor/WGF	11.8±0.2	6.0±0.2	42.2±0.5	20.4±0.4	7.0±0.9
19% PF	11.8±0.1	13.0±0.0	32.4±0.1	20.0±0.2	9.0±1.0
19% PGF	10.3±0.3	3.4±0.0	45.3±2.8	22.7±1.0	3.6±0.0

[1] Means ± standard deviation based upon four determinations. Values are on as is basis.

Table 3.5. Distribution of the four major protein classes in 19% pearling fractions of Candle and Condor barley.

Protein classes	Protein distribution (% w/w of total in WGF)					
	Candle			**Condor**		
	WGF	**19% PF**	**19% PGF**	**WGF**	**19% PF**	**19% PGF**
Albumin	(100)	35.3	51.5	(100)	29.9	60.7
Globulin	(100)	50.6	51.0	(100)	64.5	39.0
Hordein	(100)	23.1	72.7	(100)	23.1	74.9
Glutelin	(100)	32.9	70.3	(100)	29.5	77.6

pearling of grain. Bhatty (1997) reported that in hull-less barley (Condor), 30% pearling constituted true bran, which also includes the germ that is high in albumins and globulins. Therefore, 19% pearling may have been insufficient to extract the embryo, resulting in a lower level of water- and salt-soluble proteins in 19% PF.

3.3.5. Amino acid analysis

Amino acid profiles of WGF, 19% PF and 19% PGF of Candle and Condor barley are presented in Table 3.6. Candle WGF has higher concentrations of all essential amino acids (EEA) as compared to Condor WGF. In Condor WGF, the glutamic acid content was 27.9 g/16 g N, which is consistent with the higher hordein content in Condor as compared to Candle. In 19% PF of Candle, majority of the EEA decreased as compared to

Table 3.6. Amino acid composition[1] of whole grain flour and 19% pearling fractions of Candle and Condor barley.

Amino acid	**Amount (g/16 g N)**					
	Candle			**Condor**		
	WGF	**19% PF**	**19% PGF**	**WGF**	**19% PF**	**19% PGF**
Cysteine	3.4	3.2	3.6	2.9	2.6	3.1
Aspartic acid	5.8	7.6	6.1	5.6	6.8	4.7
Glutamic acid	24.6	21.9	29.4	27.9	22.4	27.9
Serine	3.8	4.2	4.2	3.9	3.7	3.8
Histidine	2.2	2.6	2.1	2.2	2.3	1.9
Glycine	4.2	4.6	3.4	3.2	4.0	2.9
*Threonine	3.7	3.2	2.8	2.7	2.7	2.4
*Methionine	2.7	1.9	1.7	1.8	1.6	1.4
Arginine	4.5	5.1	3.7	4.0	4.9	3.2
Alanine	4.4	4.7	3.8	3.3	4.1	2.8
Tyrosine	2.9	3.3	2.8	2.7	3.1	2.3
*Valine	5.4	5.2	5.0	4.4	4.8	3.9
*Phenylalanine	5.6	4.7	5.5	4.9	5.0	4.8
*Isoleucine	4.1	3.7	3.9	3.3	3.5	3.0
*Leucine	7.0	7.4	7.8	6.6	6.8	6.0
*Lysine	3.6	3.5	2.9	2.6	3.4	2.2

*Essential amino acids.

[1] Values are means of two determinations.

WGF. In fact, leucine and phenylalanine were higher in 19% PGF as compared to 19% PF. The glutamic acid content in Candle 19% PGF was highest (29.4 g/16 g N) and in both varieties, the lysine content of 19% PGF was lowest (2.2-2.9 g/16 g N). In Condor, the opposite trend was seen in 19% PF where all EAA increased as compared to WGF with lysine increasing by 29%.

Amino acid analysis of protein classes (albumins, globulins, hordeins and glutelins) extracted from WGF, 19% PF and 19% PGF of Condor and Candle are highlighted in Tables 3.7, 3.8 and 3.9, respectively. In Candle, all four protein classes had substantially higher EAA in 19% PGF as compared to WGF and 19% PF. With the exception of lysine, which was highest in the protein fractions of 19% PF, in Condor, the EAA were more

Table 3.7. Amino acid composition[1] of albumins (Alb), globulins (Glob), hordeins (Hord) and glutelins (Glut) in whole grain flour (WGF) of Candle and Condor barley.

Amino acid	Amount (g/16 g N)							
	Candle/WGF				Condor/WGF			
	Alb	Glob	Hord	Glut	Alb	Glob	Hord	Glut
Cysteine	5.4	3.6	3.1	2.3	4.8	2.9	2.8	2.1
Aspartic acid	9.3	8.5	2.0	8.4	9.0	8.3	2.3	7.2
Glutamic acid	13.2	13.2	42.5	19.3	13.0	11.0	42.9	17.7
Serine	4.0	3.9	3.1	4.5	4.0	3.3	3.1	4.4
Histidine	2.2	2.6	1.4	2.8	2.2	1.9	1.7	2.9
Glycine	6.4	5.6	2.0	6.6	6.1	5.5	1.6	6.0
*Threonine	4.8	4.1	2.8	5.1	4.6	3.6	2.3	4.6
*Methionine	2.8	2.6	1.8	2.8	2.7	2.3	1.6	2.6
Arginine	7.2	10.1	3.3	6.6	7.8	8.3	3.1	6.5
Alanine	6.2	5.9	1.9	5.5	6.3	5.4	1.5	5.3
Tyrosine	1.5	3.0	0.0	1.1	2.1	2.6	0.0	0.9
*Valine	6.9	6.4	5.3	7.4	7.1	6.1	4.7	6.6
*Phenylalanine	3.8	4.3	8.5	5.9	3.8	3.9	7.9	5.4
*Isoleucine	3.9	3.9	4.9	4.8	4.1	3.6	4.5	4.2
*Leucine	8.5	8.9	9.1	9.6	8.4	8.2	8.5	9.3
*Lysine	5.4	6.6	0.8	4.9	5.9	5.5	0.8	4.8

*Essential amino acids.

[1] Values are means of two determinations.

randomly distributed in all four protein classes of WGF, 19% PF and 19% PGF. In Candle, the globulin protein fraction in WGF and 19% PGF had higher lysine content as compared to albumin and glutelin fractions, whereas in 19% PF, the glutelin protein fraction had higher lysine as compared to albumin and globulin fractions. In Condor however the lysine contents of albumin and globulin fractions were similar to each other but higher than that in the glutelin fraction of WGF, 19% PF and 19% PGF. Overall, high glutamic acid, low aspartic acid, histidine, glycine, methionine, alanine, tyrosine and lysine characterized the hordein protein fractions of Candle and Condor barley. Albumins and globulins were high in aspartic acid, glutamic acid, glycine, arginine, valine, leucine and lysine with glutelins having an intermediate amino acid profile to that of albumins and

Table 3.8. Amino acid composition[1] of albumins (Alb), globulins (Glob), hordeins (Hord) and glutelins (Glut) in 19% pearling flour (PF) of Candle and Condor barley.

Amino acid	Amount (g/16 g N)							
	Candle/19% PF				Condor/19% PF			
	Alb	Glob	Hord	Glut	Alb	Glob	Hord	Glut
Cysteine	2.7	0.9	2.2	1.3	4.2	1.8	3.2	2.0
Aspartic acid	5.7	4.4	2.8	6.2	9.4	8.5	1.9	8.4
Glutamic acid	7.2	6.9	28.2	12.4	13.4	13.8	44.2	18.0
Serine	2.4	2.1	2.4	3.7	3.9	3.8	3.5	4.6
Histidine	1.3	1.5	1.2	2.7	2.5	2.4	1.8	3.4
Glycine	3.4	2.8	0.9	4.5	7.0	6.3	1.9	6.6
*Threonine	2.3	1.7	1.2	3.0	5.4	3.9	2.2	5.0
*Methionine	1.2	1.0	0.9	2.1	2.7	2.2	1.7	3.0
Arginine	2.9	3.8	1.6	5.2	7.7	10.6	3.1	8.5
Alanine	3.2	2.7	1.1	4.5	7.1	5.6	1.8	6.3
Tyrosine	1.7	1.6	0.4	2.6	3.1	2.9	0.0	3.0
*Valine	2.9	2.8	2.7	4.6	6.4	6.7	4.6	7.4
*Phenylalanine	1.7	2.1	4.3	3.8	3.2	4.5	7.2	5.5
*Isoleucine	1.9	1.9	2.6	3.0	4.0	4.1	4.3	4.6
*Leucine	3.4	3.6	4.9	6.5	7.3	8.7	8.0	10.8
*Lysine	2.9	2.7	0.4	3.5	6.8	6.4	0.8	5.5

*Essential amino acids.

[1] Values are means of two determinations.

globulins as compared to hordeins. For WGF, the findings of this study were consistent with those of Rhodes and Gill (1980) who reported that compared to whole seed barley proteins, the isolated albumin and globulin fractions had higher concentrations of lysine, threonine, methionine and valine.

Ingversen and Koie (1973) have reported that the distribution of lysine in salt-soluble proteins (albumins and globulins) of Emir barley (whole grain) was 44% followed by glutelin (38%) and hordeins contained the least amount of lysine with values reaching only 9%. In this study, in Condor WGF, the distribution of lysine was higher (49.9%) in storage proteins as compared to salt-soluble proteins (39.3%) (Table 3.10). Lysine distribution in salt-soluble proteins of Condor increased from 39.3% in WGF to 48.3% in

Table 3.9. Amino acid composition[1] of albumins (Alb), globulins (Glob), hordeins (Hord) and glutelins (Glut) in 19% pearled grain flour (PGF) of Candle and Condor barley.

Amino acid	Amount (g/16 g N)							
	Candle/19% PGF				Condor/19% PGF			
	Alb	Glob	Hord	Glut	Alb	Glob	Hord	Glut
Cysteine	6.6	6.9	3.8	2.2	5.9	5.2	2.5	1.9
Aspartic acid	8.7	11.7	2.3	8.9	10.0	7.9	1.5	4.0
Glutamic acid	16.3	18.2	58.6	24.8	14.4	10.4	38.8	18.9
Serine	5.8	6.6	5.7	6.3	4.3	2.6	2.8	4.0
Histidine	2.4	2.4	2.4	3.0	2.4	2.1	1.6	2.5
Glycine	8.7	10.8	3.5	8.4	7.0	5.3	1.3	6.4
*Threonine	5.4	6.6	3.4	6.1	4.4	3.4	2.0	4.8
*Methionine	4.7	4.9	2.8	3.8	2.9	2.4	1.6	2.8
Arginine	10.7	15.4	5.6	7.6	6.7	7.2	2.5	4.9
Alanine	8.8	9.8	3.1	7.5	6.0	4.7	1.5	5.0
Tyrosine	5.7	5.3	0.8	0.8	0.8	2.8	0.4	0.7
*Valine	9.4	9.1	6.3	8.1	6.9	4.7	4.9	5.6
*Phenylalanine	6.1	6.6	10.6	7.0	3.8	3.0	8.6	4.4
*Isoleucine	5.3	5.4	6.7	5.5	4.0	2.5	4.5	3.9
*Leucine	12.9	14.4	14.0	12.7	8.1	6.8	7.7	8.6
*Lysine	7.1	9.7	1.3	6.2	5.3	5.3	0.7	4.2

*Essential amino acids.

[1] Values are means of two determinations.

Table 3.10. Distribution of lysine in protein fractions of Candle and Condor WGF, 19% PF and 19% PGF.

Protein fraction	% Lysine distribution (w/w)					
	Candle			Condor		
	WGF	19% PF	19% PGF	WGF	19% PF	19% PGF
Albumin	18.3	11.1	23.9	26.6	23.8	24.7
Globulin	14.5	9.6	21.0	12.7	24.5	8.0
Hordein	7.8	2.8	18.7	12.5	7.8	13.9
Glutelin	29.1	21.6	49.7	37.4	33.0	43.2

19% PF and decreased to 32.7% in 19% PGF. The distribution of lysine was highest in the storage proteins (57.1%) of 19% PGF of Condor. In 19% PGF of Condor, 43.2% of the total lysine content was found in the glutelin fraction. Lysine distribution in hordeins of

19% PGF increased to 13.9% as compared to 12.5% in WGF but was lowest in the hordeins of 19% PF (7.8%).

In Candle, lysine distribution in 19% PF decreased in all four protein fractions and increased in 19% PGF as compared to WGF. Lysine distribution in storage proteins was highest (68.4%) in 19% PGF and lowest for salt-soluble proteins (20.7%) in 19% PF. Similar to Condor, glutelins in 19% PGF of Candle contained 49.7% of the lysine content. The distribution of lysine in the hordein fraction of 19% PGF increased to 18.7% as compared to 7.8% in WGF, but the lysine content was lowest (2.8%) in the hordein fraction of 19% PF.

Table 3.11 highlights the chemical scores of wheat (whole grain), soy protein isolate and the three flour fractions of Candle and Condor barley. Chemical scores were determined using egg proteins as a reference and are based upon the lowest ratio of essential amino acid present, which is denoted as "first limiting". Even though soy protein isolate is very high in lysine, it is limiting in methionine and cysteine, thus its chemical score of 40 is identical to that of wheat which is limiting in lysine.

Candle WGF and 19% PF have similar chemical scores ranging between 55-58. The chemical score for Candle 19% PGF was lowest (45) as compared to WGF and 19% PF, but was still higher than that of whole grain wheat and soy protein isolate. For Condor, the chemical score of 19% PF increased from 41 to 53 as compared to WGF and decreased to 35 in 19% PGF. In Candle, lysine remained the first limiting amino acid for all three flour fractions, whereas in 19% PF of Condor, both lysine and isoleucine were first limiting. The second limiting amino acid for both varieties was either isoleucine or threonine.

Table 3.11. Essential amino acid content and chemical scores[1] of whole egg[2], whole-wheat grain[2], soy protein isolate[3], and Candle and Condor WGF, 19% PF and 19% PGF.

Essential amino acid	Whole egg	Soy protein isolate	Whole wheat grain	Candle			Condor		
				WGF	19% PF	19% PGF	WGF	19% PF	19% PGF
Histidine	2.4	2.3	1.9	2.3	2.6	2.1	2.2	2.3	1.9
Isoleucine	6.6	4.3	4.0	4.1	3.7	3.9	3.3	3.5	3.0
Leucine	8.8	7.4	6.3	7.1	7.4	7.8	6.6	6.8	6.0
Lysine	6.4	6.4	2.6	3.7	3.5	2.9	2.6	3.4	2.2
Methionine + cysteine	5.5	2.2	3.5	6.1	5.1	5.3	4.6	4.2	4.5
Phenylalanine + tyrosine	10.1	8.0	8.1	8.5	8.0	8.3	7.6	8.0	7.2
Threonine	5.0	3.6	2.7	3.7	3.2	2.8	2.7	2.7	2.4
Valine	7.4	4.3	4.3	5.4	5.2	5.0	4.4	4.8	3.9
Chemical score	100	40	40	58	55	45	41	53	35
First limiting amino acid	-	Methionine + cysteine	Lysine	Lysine	Lysine	Lysine	Lysine	Isoleucine + lysine	Lysine

[1] EAA's of egg is used for reference. Chemical score = (First limiting AA/Corresponding AA from egg) x 100. i.e., for wheat, Chemical score = (2.6/6.4) x 100 = 40 (Harper, 1977).

[2] Adapted from Harper (1977).

[3] Adapted from Del Valle (1981).

The amino acid composition of a specific barley variety and the extent of pearling may affect the distribution of certain essential amino acids within the pearling fractions and thus affecting their overall nutritional value.

3.4. CONCLUSIONS

Pearling is an effective technique to fractionate barley grain to concentrate specific components in either the pearling flour or the pearled grain. In this study, pearling level of 19-21% for both Candle and Condor barley varieties was found to be optimum in concentrating protein and lipids in the pearling flour and enriching β-glucan and starch in the pearled grain. The distribution of protein classes within a barley grain, which may be dependent on variety, affects the amino acid profile of the pearling fractions. It was shown that water- and salt-soluble proteins (albumins and globulins) are not limited to the aleurone (bran) alone, whereas storage proteins (hordeins and glutelins) mostly occur (>70%) in the endosperm.

The 19% PGF in both Candle and Condor was high in glutamic acid and low in lysine, which is characteristic of hordein proteins. In 19% PF, the concentration of EAA was either slightly decreased or increased as compared to WGF. Overall, the EAA profile of albumins, globulins, hordeins and glutelins was higher in the 19% PF of Condor as compared to WGF and 19% PGF. However, the four protein classes isolated from 19% PF had lower EAA profiles as compared to WGF and 19% PGF of Candle.

3.5. REFERENCES

AACC. 1982. *Approved methods of the American Association of Cereal Chemists*, 7th ed. The Association, St. Paul, Minnesota.

AOAC. 1999. *Official methods of analysis*, 16th ed. Association of Official Analytical Chemists, Washington, D.C.

Bhatty, R.S. 1986. The potential of hull-less barley-A review. Cereal Chem. 63(2): 97-103.

Bhatty, R.S. 1992. β-Glucan content and viscosities of barelys and their roller-milled flour and bran products. Cereal Chem. 69(5): 469-471.

Bhatty, R.S. 1993. Physicochemical properties of roller-milled barley and bran flour. Cereal Chem. 70(4): 397-402.

Bhatty, R.S. 1995. Laboratory and pilot plant extraction and purification of β-glucans from hull-less barely and oat brans. J. Cereal. Sci. 22: 163-170.

Bhatty, R.S. 1997. Milling of regular and waxy starch hull-less barleys for the production of bran and flour. Cereal Chem. 74(6): 693-699.

Bhatty, R.S. 1999. The potential of hull-less barley. Cereal Chem. 76(5): 589-599.

Chung, J., and Pomeranz, Y. 1985. Proteins in developing and germinating cereal grains and grain. Ch. 6 in *Digestibility and Amino Acid Availability in Cereals and oilseeds*, J.W. Finely and D.T. Hopkins (Ed.), p. 109-168. American Association of Cereal Chemists, Inc., St. Paul, Minnesota.

Del Valle, F.R. 1981. Nutritional qualities of soya protein as affected by processing. J. Am. Oil Chem Soc. 58: 419-429.

El-Negoumy, A.M., Newman, C. W., and Moss, B.R. 1977. Chromatographic fractionation and composition of the components of the salt-soluble proteins from Hiproly (CI 3947) and Hiproly normal (CI 4362) barleys. Cereal Chem. 54(2): 333-344.

Fastnaught, C.E., Berglund, P.T., Holm, E.T., and Fox, G.J. 1996. Genetic and environmental variation in β-glucan content and quality parameters of barley for food. Crop Sci. 36: 941-946.

Gohl, B., Larsson, K., Nilsson, M., Theander, O., and Thomke, S. 1977. Distribution of carbohydrates in early harvested barley grain. Cereal. Chem. 54(3): 690-698.

Harper, A.E. 1977. Human amino acid and nitrogen requirments as the basis for evaluation of nutritional quality of proteins. Ch. 15 in *Food Proteins*, J.R. Whitaker and S.R. Tannenbaum (Ed.), p. 363-386. AVI Publishing Company, Inc., Westport, Connecticut.

Hofman, M. 1975. Proteins and amino acids of barley pearlings. Lebensm.-Wiss. u. -Technol. 8: 82-84.

Ingversen, J., and Koie, B. 1973. Lysine rich proteins in the salt-soluble protein fraction of barley. Phytochemistry. 12: 73-98.

Kirkman, M.A., Shewry, P.R., and Miflin, B. J. 1982. The effect of nitrogen nutrition on the lysine content and protein composition of barely seeds. J. Sci. Food Agric. 33: 115-127.

Klamczynski, A., Baik, B.-K., and Czuchajowska, Z. 1998. Composition, microstructure, water imbibition, and thermal properties of abraded barley. Cereal Chem. 75(5): 677-685.

Koie, B., and Nielsen, G. 1977. Extraction and separation of hordeins. Ch. 2 in *Techniques for the Separation of Barley and Maize Seed Proteins*, B.J. Miflin and P.R. Shewry (Ed.), p. 25-35. Commission of the European Communities, Luxembourg, Belgium.

Lasztity, R. 1984. Barley Proteins. Ch. 5 in *The Chemistry of Cereal Proteins*, R. Lasztity (Ed.), p. 117-130. CRC Press, Inc., Boca Raton, Florida.

Linko, R., Lapvetelainen, A., Laakso, P., and Kallio, H. 1989. Protein composition of a high-protein barley flour and barley grain. Cereal Chem. 66(6): 478-482.

MacGregor, A.W., and Fincher, G.B. 1993. Carbohydrates of the barley grain. Chap. 3 in *Barley: Chemistry and Technology*, A.W. MacGregor and R.S. Bhatty (Ed.), p. 73-130. The American Association of Cereal Chemists, Inc., St. Paul, Minnesota.

McCleary, B.V., and Glennie-Holmes, M. 1985. Enzymatic quantification of (1→3), (1→4)-ß-D-glucan in barley and malt. J. Inst. Brew. 91: 285-295.

McCleary, B.V., Sloah, V., and Gibson, T.S. 1994. Quantitative measurement of total starch in cereal flours and products. J. Cereal Science. 20: 51-58.

Millet, M.O., Montembault, A., and Autran, J.C. 1991. Hordein composition differences in various anatomical regions of the kernel between two different barley types. Sciences Des Aliments. 11: 156-161.

Munck, L. 1981. Barley for food, feed and industry. Ch. 21 in *Cereals: A Renewable Resource, Theory and Practice*, Y. Pomeranz and L. Mu[illegible]ck (Ed.), p. 427-461. The American Association of Cereal Chemists, Inc., St Paul, Minnesota.

Peterson, D.M. 1994. Barley tocols: Effects of milling, malting, and mashing. Cereal Chem. 71(1): 42-44.

Pomeranz, Y. 1974. Food uses of barley. Crit. Rev. Food Technol. January: 377-394.

Pomeranz, Y. 1987. Composition. Ch. 4 in *Modern Cereal Science and Technology*, Y. Pomeranz (Ed.), p. 40-53. VCH Publishers, Inc., New York, New York.

Rhodes, A. P., and Gill, A.A. 1980. Fractionation and amino acid analysis of the salt-soluble protein fractions of normal and high-lysine barleys. J. Sci. Food Agric. 31: 467-473.

Robbins G.S., and Pomeranz, Y. 1972. Composition and utilization of milled barley products. III. Amino acid composition. Cereal Chem. 49(2): 240-246.

SAS Institute Inc. 1999. SAS/STAT *User's Guide*, version 8, SAS Institute, Cary, North Carolina.

Sedgwick, G.W., Fenton, T.W., and Thompson, J.R. 1991. Effect of protein precipitating agents on the recovery of plasma free amino acids. Can. J. Anim. Sci. 71: 953-957.

Shewry, P.R., Hill, J.M., Pratt, H.M., Leggatt, M.M., and Miflin, B.J. 1978. An evaluation of techniques for the extraction of hordein and glutelin from barley seed and a comparsion of the protein composition of Biomi and Riso 1508. J. Exp. Bot. 29(110): 677-692.

Sumner, A.K., Gebre-Egziabher, A., Tyler, R.T., and Rossnagel, B.G. 1985. Composition and properties of pearled and fines fractions from hulled and hull-less barley. Cereal Chem. 62(2): 112-116.

Wu, Y.V., Stringfellow, A.C., and Inglett, G.E. 1994. Protein and β-glucan enriched fractions from high-protein, high β-glucan barleys by sieving and air classification. Cereal Chem. 71(3): 220-223.

Yupsanis, T., Burgess, S.R., Jackson, P.J., and Shewry, P.R. 1990. Characterization of the major protein component from aleurone cells of barley (*Hordeum vulgare* L.). J. Exp. Bot. 41(225): 385-392.

Zheng, G.H., Rossnagel, B.G., Tyler, R.T., and Bhatty, R.S. 2000. Distribution of β-glucan in the grain of hull-less barley. Cereal Chem. 77(2): 140-144.

4. EFFECT OF ALKALINE EXTRACTION ON THE RECOVERY AND PURITY OF PROTEINS, β-GLUCAN AND STARCH IN HULL-LESS BARLEY WHOLE GRAIN AND PEARLING FLOURS[1]

4.1. INTRODUCTION

Commercial pearling of barley results in the manufacturing of two main products, the pearling flour (bran) and pearled grain, which can also be milled into flour or used as is. Depending on the level of pearling, this process basically separates and concentrates barley grain components in the pearling fractions. The pearling flour is essentially made up of the aleurone, subaleurone and germ tissue and is high in metabolic proteins (albumins and globulins), lipids (including tocopherols and tocotrienols) and minerals. The pearled grain flour of barley contains substantial amounts of β-glucan present in cell walls of the endosperm and starch that is embedded in a protein matrix.

Further processing such as solvent extraction and air classification can be employed on barley pearling fractions to extract and purify specific barley components. Due to the demonstrated cholesterol lowering effects of β-glucan in both animals (chicks, rats, pigs and hamsters) and humans (Newman and Newman 1991; Malkki et al 1992), interest has focused mainly on the extraction of this hydrocolloid. During the β-glucan extraction process, proteins are precipitated out and treated as a by-product. Thus, proteins have received little attention with regards to their nutritional quality and functionality.

Using 20% sodium carbonate, Temelli (1997) obtained β-glucan gum from whole grain barley flour with high purity (89%) and recovery (87%) between pH 7-8 and 55°C. But at higher pH's (9-10) and temperatures of 50-55°C, the β-glucan gum was highly contaminated with protein (27-31%) due to their increased solubilization. Bhatty (1995) extracted β-glucan using 1*M* sodium hydroxide (NaOH) from barley and oat bran with a yield of 69-76% whereas Dawkins and Nnanna (1993) obtained oat bran β-glucan with 70-89% purity at pH's between 9.2-10.5 and temperatures of 50-55°C using 2*M* NaOH with a recovery of 66-91% of total bran proteins. Palmer (1986) purified barley β-glucan gum using 4% NaOH followed by neutralization and dialysis, without the precipitation of proteins. Wu et al (1994) obtained barley flour with 28% β-glucan by sieving and air classification. This flour fraction was also high in protein (21%) and fat (5%) and lower in starch (21%). Wood et al (1978) have shown that flour particle size, pH, temperature, and ionic strength of extraction media all influence β-glucan yields and affect both protein functionality and starch integrity.

---

[1] A version of this chapter is to be submitted to Cereal Chemistry for consideration for publication.

Soy protein market has grown considerably in recent years due to the nutritive quality and functional properties of soy proteins. Due to the high cost of animal proteins and the inexpensive production of cereal grain proteins (Wu and Sexon 1975), there is a growing demand for new protein ingredients with favorable taste (Cluskey et al 1973). The quality of foods can be nutritionally improved by supplementation with proteins and other such components. Cluskey et al (1976) formulated a milk-like breakfast drink and a flavored acidic type beverage using oat protein concentrate from whole grain oat flour.

Depending upon the cereal variety, protein extraction is greatly dependent on solvent type, solvent concentration and pH, isoelectric pH and solvent-to-feed ratio. Alkaline extraction of protein concentrates from whole grain barley (Wu et al 1979), wheat (Wu and Sexon 1975), oat (Cluskey et al 1973), corn (Wu and Sexon 1976), triticale (Wu et al 1976) and rice bran (Chen 1970) has been reported. The most commonly used solvent for protein extraction is NaOH of varying concentrations at pH between 9-12. Isoelectric pH used for the precipitation of proteins is in the range of 4.5-5.5 with a solvent-to-feed ratio between 7:1 to 15:1. Based on the extraction conditions used, variation exists in both protein yield and purity.

Pearling of barley grain causes the distribution of protein and other major components (i.e. β-glucan, starch and lipids) within the pearling fractions. It appears that both barley β-glucan and proteins are governed by similar extraction parameters. It would be economically advantageous if a single solvent extraction procedure can be developed to isolate these barley constituents with minimal structural degradation. However, simultaneous recovery and evaluation of the properties of protein, β-glucan and starch fractions have not been reported. As well, the effects of alkaline extraction on protein, β-glucan and starch recovery from whole grain, pearling flour and pearled grain flour of different barley varieties have not been examined. Therefore, the objectives of this study were:

1. to study the effect of extraction conditions on the isolation of barley protein concentrates from whole grain flour (WGF), pearling flour (19% PF) and pearled grain flour (19% PGF) for maximizing protein purity and yield,
2. to determine the extraction conditions for the separation of 19% PGF into β-glucan, protein and starch fractions by maximizing the yield and purity of each fraction, and

3. to assess the nutritional quality of the barley protein concentrates obtained by evaluating their amino acid profile.

4.2. MATERIALS AND METHODS

4.2.1. Materials

CDC Candle (hull-less waxy) and Condor (hull-less regular) barley grains (200 kg obtained from the sources indicated in Section 3.2.1) were pearled at the Center for Agri-Industrial Technology as described in Chapter 3 (Section 3.2.2). Whole grain and 19% pearled grain were milled into flour using a hammer mill fitted with a 0.5 mm screen prior to extraction.

4.2.2. Extraction conditions

Protein isolates, β-glucan gum and starch residue from WGF, 19% PF and 19% PGF of both Candle and Condor barley were obtained by alkali extraction (Fig. 4.1). Solvents, consisting of 0.035*M* sodium hydroxide (NaOH) or sodium carbonate (Na_2CO_3) with pH adjustment to 10 or 11 were prepared and extraction temperatures of 23°C (ambient temperature), 40°C or 50°C were used. Flour samples (100 g) were dispersed in 20 volumes of appropriate solvent and pH was re-adjusted accordingly and maintained for the entire extraction period of 1.5 hr with constant stirring. The flour slurry was centrifuged (Model J2-21, Beckman Instrument Co., Mississauga, ON) for 30 min at 18,000 x *g* at 23°C to remove solids (starchy residue). The starchy residue was re-dissolved in distilled water, neutralized using 2*M* HCl and then freeze-dried.

The supernatant was decanted, pH was adjusted to 5.5 (modification from pH 4.5 as reported by Wood et al (1978), Wood et al (1989), Bhatty (1995) and Temelli (1997))

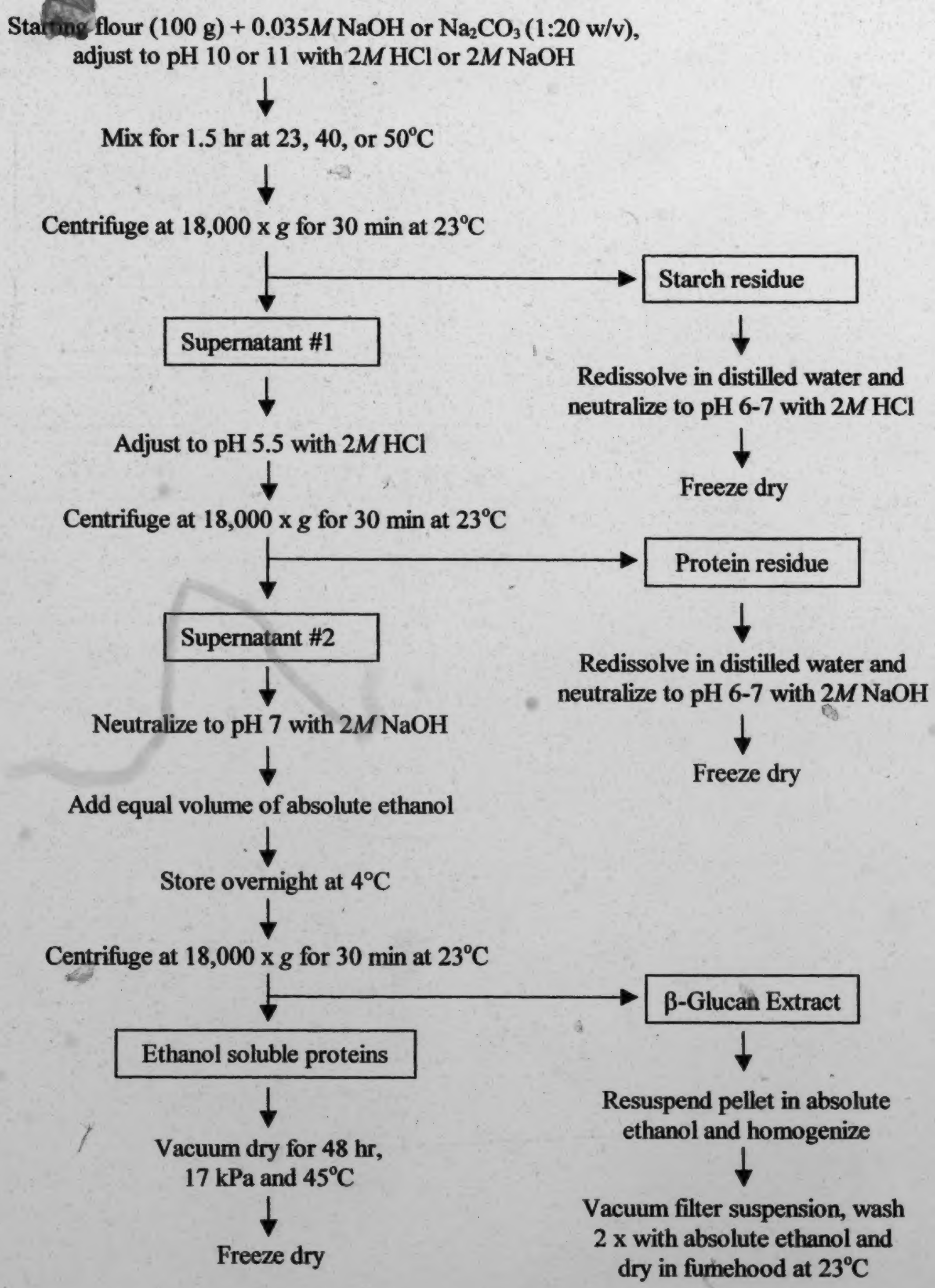
Starting flour (100 g) + 0.035*M* NaOH or Na_2CO_3 (1:20 w/v), adjust to pH 10 or 11 with 2*M* HCl or 2*M* NaOH
Mix for 1.5 hr at 23, 40, or 50°C
Centrifuge at 18,000 x *g* for 30 min at 23°C
Starch residue
Supernatant #1
Redissolve in distilled water and neutralize to pH 6-7 with 2*M* HCl
Freeze dry
Adjust to pH 5.5 with 2*M* HCl
Centrifuge at 18,000 x *g* for 30 min at 23°C
Protein residue
Supernatant #2
Redissolve in distilled water and neutralize to pH 6-7 with 2*M* NaOH
Freeze dry
Neutralize to pH 7 with 2*M* NaOH
Add equal volume of absolute ethanol
Store overnight at 4°C
Centrifuge at 18,000 x *g* for 30 min at 23°C
β-Glucan Extract
Ethanol soluble proteins
Resuspend pellet in absolute ethanol and homogenize
Vacuum dry for 48 hr, 17 kPa and 45°C
Vacuum filter suspension, wash 2 x with absolute ethanol and dry in fumehood at 23°C
Freeze dry

Figure 4.1. Flow diagram for the extraction of starch, protein and β-glucan from whole grain and pearling flours of Candle and Condor barley.

with 2*M* HCl to precipitate the proteins followed by centrifugation (30 min at 18,000 x *g* at 23°C). The precipitated proteins were re-dissolved in distilled water, neutralized using 2*M* NaOH and then freeze-dried. The supernatant was decanted and an equal volume of absolute ethanol was added to obtain a 50% ethanol solution that was left undisturbed overnight at 4°C to precipitate β-glucan. The following day, β-glucan solution was centrifuged (30 min at 18,000 x *g* at 23°C) and the rubbery pellet was suspended in 400-500 mL absolute ethanol which was then homogenized (16,200 rpm, Polytron homogenizer, Brinkmann Instruments, Rexdale, ON) at ambient temperature to obtain a uniform dispersed solution. The β-glucan suspension was vacuum filtered (Whatman #2 filter paper), washed twice with 100-200 mL absolute ethanol and was left under vacuum filtration for 15-20 min. The β-glucan residue was gently scraped off the filter paper and further air dried in a fume hood (23°C) overnight. β-Glucan residue was pulverized using a mortar and pestle and stored at 4°C until analysis. The 50% ethanol solution (supernatant) obtained after β-glucan precipitation was vacuum dried (48 hr, 17 kPa and 45°C) to 40-45% of its original volume and then freeze-dried. Both the starch and protein residues (isoelectric-precipitated proteins and ethanol-soluble proteins) collected after freeze drying were ground in a coffee grinder to obtain a powdery material and stored at 4°C until analysis.

4.2.3. Compositional analysis

Compositional analysis was carried out on both Candle and Condor WGF, 19% PF and 19% PGF. Protein content was determined on the protein extracts from each of the three flour fractions. Protein, β-glucan and starch contents were determined on the respective extracts obtained from Candle and Condor 19% PGF. β-Glucan, starch ash, moisture, lipid and protein contents of samples were determined according to standard protocols indicated in Chapter 3 (Section 3.2.3).

4.2.4. Amino acid analysis

Amino acid profile of the protein concentrates extracted from WGF, 19% PF and 19% PGF, from both Candle and Condor barley, was determined according to the procedure outlined in Chapter 3 (Section 3.2.7).

4.2.5. Statistical analysis

Extraction of protein concentrates from Candle and Condor barley WGF, 19% PF and 19% PGF at each pH/solvent combination was carried out in duplicate. Extraction of protein, β-glucan and starch from 19% PGF of Candle and Condor barley at each pH/solvent/temperature combination was also done in duplicate. Compositional analyses, amino acid composition and color value results are averages of duplicate determinations on each sample. Analysis of variance of results was done using General Linear Model (GLM) procedure of SAS Statistical Software, Version 8 (SAS Institute, 1999). The model consisted of the main effects of flour type, pH and solvent and pH*solvent interaction effect on protein yield and purity. Multiple comparison of means was performed by Tukey's studentized range (HSD) test at $\alpha=0.05$ level.

4.3. RESULTS AND DISCUSSION

4.3.1. Compositional analysis of barley pearling fractions

As was also demonstrated in Chapter 3, Candle 19% PF had increased protein (25.0%), lipid (9.6%) and ash (6.0%) contents as compared to WGF whereas 19% PGF was enriched in β-glucan (8.4%) and starch (71.8%) (Table 4.1). The protein content of

Table 4.1. Proximate analysis of Candle and Condor barley whole grain and 19% pearling flour fractions obtained from large batch of pilot processing.

Analysis	% (w/w, dry matter basis)					
	Candle			Condor		
	WGF	19% PF	19% PGF	WGF	19% PF	19% PGF
Starch	61.1	18.8	71.8	59.4	19.0	70.0
Protein	14.9	25.0	12.1	18.5	29.2	15.9
β-Glucan	7.5	4.9	8.4	6.4	5.8	7.1
Lipid	2.7	9.6	1.3	2.2	9.0	1.3
Ash	2.1	6.0	1.0	1.9	5.2	1.0

19% PGF (12.01%) was similar but slightly lower than that of WGF (14.9%). Similar trends were seen in Condor with the exception of β-glucan, whose distribution in WGF (6.4%), 19% PF (5.8%) and 19% PGF (7.1%) seemed to be more uniform as compared to that in Candle. In terms of varietal differences, Condor barley WGF and pearling fractions were higher in protein whereas Candle was higher in β-glucan, lipids and ash with starch content being similar in both varieties.

4.3.2. Extraction conditions

4.3.2.1. Protein recovery and purity

For Candle barley, maximum protein recovery ($P \leq 0.05$) was achieved at pH 11/23°C using 0.035*M* NaOH for WGF, 19% PF and 19% PGF (Table 4.2). Protein recovery values for the three flour fractions were higher (66-74%) when NaOH was used compared to 56-69% achieved with 0.035*M* Na_2CO_3 at pH 11/23°C. In terms of the amount of protein extracted, 19% PF consistently resulted in the highest yield at all extraction conditions but its purity was lowest, whereas the exact opposite was true for 19% PGF. Protein purity was highest at pH 11 for both solvents, with NaOH resulting in

Table 4.2. Amount of protein extracted, purity and recovery of proteins using 0.035*M* NaOH and Na_2CO_3 at pH 10 and 11 at 23°C for Candle whole grain and pearling flour fractions.

Variety & flour fraction	Wt. of extract (g/100 g)	Protein content (% w/w)	Wt. of protein (g)	% Protein recovery
Candle			NaOH, pH 10	
WGF	6.3	68.8	4.3	32.3±1.7^{f}
19% PF	12.8	64.8	8.3	36.6±1.4^{e}
19% PGF	5.1	77.0	3.9	36.0±1.8^{e}
Candle			NaOH, pH 11	
WGF	11.0	80.3	8.9	66.1±0.3^{b}
19% PF	21.5	70.2	15.1	66.5±1.3^{b}
19% PGF	9.8	82.3	8.0	74.2±2.2^{a}
Candle			Na_2CO_3, pH 10	
WGF	5.4	65.0	3.5	26.1±0.6^{g}
19% PF	12.0	60.2	7.2	31.8±0.3^{f}
19% PGF	3.2	62.0	2.0	18.3±2.9^{h}
Candle			Na_2CO_3, pH 11	
WGF	9.9	72.6	7.6	56.5±2.7^{d}
19% PF	20.5	66.5	13.6	60.1±0.6^{c}
19% PGF	9.5	78.9	7.5	69.0±2.6^{b}

$^{a-h}$Means with different letters within a column are significantly different ($P \leq 0.05$). Values are on a as is basis.

higher purity protein extracts (70-82%) than Na_2CO_3 (66-79%) for the three flour fractions.

For Condor barley, protein recovery was highest ($P \leq 0.05$) at pH 11 for both solvents and NaOH resulted in significantly higher ($P \leq 0.05$) recoveries (68-74%) than Na_2CO_3 (60-63%) for WGF, 19% PF and 19% PGF (Table 4.3). As with Candle, Condor 19% PF yielded a higher amount of protein at all pH and solvent conditions with values being the highest at pH 11 (16-18 g). Purity of proteins was highest for 19% PGF at all extraction conditions with values reaching 92% using NaOH at pH 11/23°C. Overall, the protein concentrates obtained from Condor flour samples (WGF, 19% PF and 19% PGF)

Table 4.3. Amount of protein extracted, purity and recovery of proteins using 0.035*M* NaOH and Na_2CO_3 at pH 10 and 11 at 23°C for Condor whole grain and pearling flour fractions.

Variety & flour fraction	Wt. of extract (g/100 g)	Protein content (% w/w)	Wt. of protein (g)	% Protein recovery
Condor			**NaOH, pH 10**	
WGF	5.6	78.8	4.4	26.8 ± 1.0^{f}
19% PF	11.9	67.0	8.0	30.5 ± 0.6^{e}
19% PGF	4.4	88.6	3.8	27.0 ± 3.3^{f}
Condor			**NaOH, pH 11**	
WGF	13.4	87.8	11.7	70.8 ± 0.2^{b}
19% PF	23.9	75.0	17.9	68.5 ± 0.4^{b}
19% PGF	11.5	92.0	10.6	73.9 ± 0.11^{a}
Condor			**Na_2CO_3, pH 10**	
WGF	4.5	70.0	3.4	20.4 ± 0.0^{g}
19% PF	12.4	65.9	8.2	31.2 ± 0.6^{e}
19% PGF	3.5	75.6	2.6	18.3 ± 0.4^{g}
Condor			**Na_2CO_3, pH 11**	
WGF	12.3	83.9	10.3	62.3 ± 1.0^{cd}
19% PF	22.3	70.3	15.7	60.0 ± 0.2^{d}
19% PGF	10.4	86.1	9.0	63.1 ± 0.7^{c}

$^{a-g}$Means with different letters within a column are significantly different ($P \leq 0.05$). Values are on a as is basis.

had higher protein purity (66-92%) at all extraction conditions examined as compared to those from Candle (60-82%).

For Candle, increasing the extraction (0.035*M* NaOH, pH 11) temperature of 19% PGF from 23°C to 40°C resulted in lower protein purity (76%), but a larger amount of extract (10 g) was obtained, thus no significant difference in protein recovery was evident (Table 4.4). At 50°C, protein recovery dropped to 69% as compared to 74% at 23°C extraction. Increasing the protein extraction temperature to 40°C and 50°C resulted in a

Table 4.4. Amount of protein extracted, purity and recovery of proteins using 0.035*M* NaOH and Na_2CO_3 at pH 11 and at 23, 40 and 50°C for Candle and Condor 19% PGF.

Sample	Solvent	pH	Temp (°C)	Wt. of extract (g/100 g)	Protein content (% w/w)	Wt. of protein (g)	% Protein recovery
Candle: 19% PGF	NaOH	11	23	9.8	82.3	8.0	74.2±2.2
	NaOH	11	40	10.5	76.4	8.0	73.7±0.6
	NaOH	11	50	9.1	77.0	7.6	69.6±0.8
	Na_2CO_3	11	23	9.5	78.9	7.5	69.0±2.6
	Na_2CO_3	11	40	9.9	78.3	7.7	71.2±0.6
	Na_2CO_3	11	50	8.9	76.0	6.8	62.8±0.4
Condor: 19% PGF	NaOH	11	23	11.5	92.0	10.6	73.9±0.1
	NaOH	11	40	12.2	86.6	10.5	73.8±0.5
	NaOH	11	50	11.4	87.7	10.0	69.9±0.2
	Na_2CO_3	11	23	10.4	86.1	9.0	63.1±0.7
	Na_2CO_3	11	40	10.1	86.6	8.7	61.2±0.5
	Na_2CO_3	11	50	10.1	86.3	8.7	61.2±2.6

Means ± standard deviation based upon four determinations. Values are on a as is basis.

lower protein purity (76-77%) as compared to extractions carried out at 23°C where protein purity was 82%. In contrast, using Na_2CO_3 (0.0350*M*, pH 11) at 40°C resulted in a slightly higher protein recovery (71%) as compared to ambient temperature extraction (69%) but decreased to 63% at 50°C. Similar results were obtained for Condor in comparison to Candle as the extraction (NaOH, pH 11) temperature was increased from 23°C to 40°C and 50°C for 19% PGF (Table 4.4). Extraction of protein concentrates using Na_2CO_3, pH 11 at 40°C and 50°C did not affect protein recovery (61%) as compared to extraction at 23°C (63%).

At pH 10, using both solvents at 23°C, over 40% (w/w) of the total proteins still remained in the starchy residue for both varieties and the three flour fractions. Overall, 8-13% of Candle and Condor barley proteins were recovered in the 50% ethanol supernatant after β-glucan precipitation, indicating that these proteins are alcohol-soluble hordeins, which did not coagulate at the isoelectric pH of 5.5. Traditionally, during the extraction of β-glucan from oat and barley grains, the proteins were precipitated out of solution at pH 4.5 (Wood et al 1978; Dawkins and Nnanna 1993; Bhatty 1995; Temelli 1997). In this study, sequential precipitation of proteins at pH levels of 5.5, 5.0 and 4.5 was evaluated in preliminary tests. For both Candle and Condor barley, adjustment to pH 5.5 coagulated 95% (w/w, of total proteins in flour), 97% and 100% of total proteins from WGF, 19% PF and 19% PGF, respectively. Based on these findings, isoelectric pH of 5.5 was adopted to obtain protein concentrates from Candle and Condor WGF, 19% PF and 19% PGF.

Feller et al (1966) recovered 66-71% of total wheat bran proteins using alkali extraction between pH 10.5-12.5 and further increased the yield by recovering an additional 11.7% of total proteins after heating the supernatant to 100°C and coagulating those proteins not precipitated out at the isoelectric point of pH 5.5. Wu et al (1979) obtained protein concentrates from high-protein and high-lysine barley varieties (WGF) using 0.045-0.06*M* NaOH at pH 11.2. Protein purity ranged from 72 to 84% with 51-72% recovery depending on the variety. Also, Cluskey et al (1976) extracted protein concentrates from dehulled oat WGF using water as the solvent with pH adjusted to 10 using 10*M* NaOH. Purity of protein concentrates was between 74-76% with a low yield (16-22%). Protein concentrates were then utilized at 4% level to fortify acidic or neutral beverages. The best extraction conditions (0.035*M* NaOH, pH 11/23°C) evaluated in this study resulted in both high protein purity (70-92%) and recovery (66-74%) for WGF and the pearling flours.

4.3.2.2. β-Glucan purity and recovery

The effect of extraction on both β-glucan and starch were evaluated using the best conditions determined for proteins. For this purpose, 19% PGF was used as the starting material because this fraction had the highest content of both β-glucan and starch for Candle and Condor barley. The effect of temperature was also determined using solvents NaOH and Na_2CO_3 at pH 11.

The amount of β-glucan extracted from Candle barley increased with temperature (23-50°C) for both solvents while β-glucan purity remained consistently around 70-72% for NaOH (pH 11) and 68-73% for Na_2CO_3 (pH 11) (Table 4.5). β-Glucan recovery was highest (66-68%) for both NaOH and Na_2CO_3 at pH 11 and 50°C. As with Candle, the amount of β-glucan extracted from Condor barley also increased with temperature for both solvents (Table 4.5). Also, for both solvents, β-glucan purity remained between 66-70% and the recovery was highest (53-61%) at pH 11 and 50°C with values being higher for NaOH. Overall, at pH 11 and 50°C, β-glucan recovery was higher for Candle as compared to that for Candle using both solvents.

Zeng et al (1998) extracted β-glucan from oat groats using boiling water and then further purified the β-glucan by treating the extract with amyloglucosidase at 50°C/30 min. The gum extract had a β-glucan content of 83-87%, protein content of 8-10% with <1% starch contamination. Beer et al (1997) compared two methods of β-glucan extraction from oat bran. Hot water extraction with termamyl treatment extracted 51-64% of total β-glucan whereas treating oat bran for 16 hr using 5% NaOH at 22°C resulted in only 31-33% β-glucan. Also, Beer et al (1996) extracted β-glucan from oat bran using water,

Table 4.5. Amount of β-glucan extracted, purity and recovery of β-glucan using 0.035*M* NaOH and Na_2CO_3 at pH 10 and 11 and at 23, 40 and 50°C for Candle and Condor 19% PGF.

Sample	Solvent	pH	Temp (°C)	Wt. of extract (g/100 g)	β-Glucan content (% w/w)	Wt. of β-glucan (g)	% β-Glucan recovery
Candle: 19% PGF	NaOH	10	23	5.3	68.0	3.6	48.4±0.8
	NaOH	11	23	5.5	72.5	4.0	53.0±2.5
	NaOH	11	40	6.7	70.3	5.0	62.5±0.8
	NaOH	11	50	7.3	70.3	5.1	68.3±1.5
	Na_2CO_3	10	23	5.4	66.3	3.6	47.5±1.9
	Na_2CO_3	11	23	5.4	69.5	3.7	49.9±0.9
	Na_2CO_3	11	40	6.1	73.5	4.5	60.2±2.1
	Na_2CO_3	11	50	7.2	68.7	5.0	66.3±0.2
Condor: 19% PGF	NaOH	10	23	2.56	64.6	1.6	25.9±0.6
	NaOH	11	23	2.9	70.0	2.0	31.5±0.9
	NaOH	11	40	3.9	69.3	2.7	42.7±0.6
	NaOH	11	50	5.4	69.8	3.8	61.0±1.6
	Na_2CO_3	10	23	2.6	64.1	1.7	26.7±0.5
	Na_2CO_3	11	23	2.8	66.0	1.9	29.3±0.6
	Na_2CO_3	11	40	3.5	69.4	2.4	37.9±1.6
	Na_2CO_3	11	50	5.0	67.6	3.4	53.2±2.9

Means ± standard deviation based upon four determinations. Values are on a as is basis.

which was adjusted to pH 10 with 20% sodium carbonate. The β-glucan was either precipitated out using 50% ethanol or obtained through dialysis against distilled water for 48 hr at 22°C. The β-glucan content using the dialysis procedure was 66% as compared to 62% using the alkaline method. Due to the extreme length of time needed for dialysis and only 4% difference in the β-glucan content, Beer et al (1996) applied the alkali extraction procedure at pilot plant level and obtained a β-glucan content of 62% with 6% starch and 4% protein, which were similar to those obtained at lab scale. Wood et al (1989) used defatted and air classified oat bran to extract β-glucan gum in water with pH adjustment to 10 using 20% Na_2CO_3 and the extract obtained had a β-glucan content of 78%.

The focus of the current study was to obtain protein, starch and β-glucan fractions of maximum yield and purity from 19% PGF using a single procedure, which would not alter the structural or functional properties of the various components. Using the protein extraction method outlined in Figure 4.1, it is possible to further purify the β-glucan fraction by subjecting the supernatant (after protein coagulation and centrifugation) to enzymatic treatment such as α-amylase (for example, heat stable amylase, Termamyl). Since the majority of the proteins and starch have already been removed, the enzymatic heat treatment will not adversely affect their composition, but result in higher purity β-glucan.

4.3.2.3. Starch purity and recovery

For Candle, starch purity was highest (85-87%) for Na_2CO_3 at pH 11 and 40/50°C and NaOH at pH 11/50°C, corresponding to 93-96% recovery (Table 4.6). Under the same conditions, starch purity of Condor was between 80-83% with 94-96% recovery. For both solvents, starch purity increased with temperature whereas the amount of starch extracted did not show any consistent trends. In general, with the exception of Candle/NaOH/pH 11/23°C, starch recovery was quite high (90-96%) for 19% PGF for both barley varieties and seemed independent of the type of solvent and pH level used. During the extraction of β-glucan concentrate from oat bran and rolled oats, Dawkins and Nnanna (1993) recovered 64% oat bran starch at extraction conditions of pH 9.2/50°C but recovered 87% starch from rolled oats at pH 8/55°C and pH 10.5/55°C.

4.3.3. Amino acid analysis

The amino acid composition of the protein concentrates obtained in this study are presented in Table 4.7, whereas that of the starting flours was reported previously in

Table 4.6. Amount of starch extracted, purity and recovery of starch using 0.035*M* NaOH and Na_2CO_3 at pH 10 and 11 and at 23, 40 and 50°C for Candle and Condor 19% PGF.

Sample	Solvent	pH	Temp (°C)	Wt. of extract (g/100 g)	Starch content (% w/w)	Wt. of starch (g)	% Starch recovery
Candle: 19% PGF	NaOH	10	23	75.4	77.7	58.6	90.6±0.8
	NaOH	11	23	70.9	78.3	55.8	86.6±2.3
	NaOH	11	40	73.5	82.1	60.3	93.4±0.8
	NaOH	11	50	70.0	85.7	60.0	93.2±0.0
	Na_2CO_3	10	23	80.9	74.9	60.6	93.6±0.9
	Na_2CO_3	11	23	74.5	78.8	59.4	92.0±0.8
	Na_2CO_3	11	40	69.9	86.4	60.4	93.6±0.8
	Na_2CO_3	11	50	71.2	86.7	61.7	95.9±0.4
Condor: 19% PGF	NaOH	10	23	78.8	74.8	59.0	94.2±0.5
	NaOH	11	23	74.6	78.0	58.2	92.7±0.8
	NaOH	11	40	75.7	77.3	58.5	93.4±3.5
	NaOH	11	50	71.2	83.2	59.2	94.7±0.5
	Na_2CO_3	10	23	85.8	69.4	59.3	94.9±0.6
	Na_2CO_3	11	23	78.2	74.7	58.4	93.4±0.9
	Na_2CO_3	11	40	72.9	80.8	58.9	94.0±0.6
	Na_2CO_3	11	50	72.0	83.3	59.9	95.8±0.5

Means ± standard deviation based upon four determinations. Values are on a as is basis.

Chapter 3 (Table 3.6). According to the FAO essential amino acid (EAA) requirements of adult humans (Cluskey et al 1976) (Table 4.7), Candle 19% PF and 19% PGF are deficient in methionine, isoleucine and lysine whereas Condor 19% PF and 19% PGF are further lower in threonine (Table 3.6). For Candle, isoelectrically (pH 5.5) precipitated proteins extracted from WGF and 19% PF had lower amino acid (AA) concentrations (Table 4.7) but the protein concentrate obtained from 19% PGF either showed no difference or had lower AA values as compared to those of the corresponding original flour samples (Table 3.6). In contrast, for Condor, with the exception of the lower lysine values in the protein concentrates obtained from WGF and 19% PF, all other EAA concentrations were higher in the protein concentrates and either met or exceeded the FAO values.

Table 4.7. Amino acid composition[1] of proteins extracted (0.035*M* NaOH, pH 11 and 23°C) from whole grain flour and 19% pearling fractions of Candle and Condor barley.

Amino acid	**FAO Pattern**[2]	**Amount (g/16 g N)**					
		Candle			**Condor**		
		WGF	**19% PF**	**19% PGF**	**WGF**	**19% PF**	**19% PGF**
Cysteine		2.0	2.2	2.6	2.6	2.2	2.4
Aspartic acid		4.0	5.0	4.1	4.6	5.0	3.7
Glutamic acid		23.4	20.6	26.8	31.8	19.5	22.3
Serine		3.2	3.4	3.6	4.1	3.8	3.8
Histidine		1.8	2.7	2.0	2.4	2.6	2.1
Glycine		3.7	3.5	3.5	3.1	4.7	3.3
*Threonine	2.8	2.8	2.5	2.9	2.6	3.6	3.1
*Methionine	2.2	2.1	1.7	1.9	1.8	2.6	2.3
Arginine		3.7	4.4	3.7	3.9	5.4	3.4
Alanine		3.0	3.3	3.2	3.0	4.1	3.0
Tyrosine		3.1	2.5	2.4	3.0	3.1	3.0
*Valine	4.2	4.4	4.1	4.9	4.4	5.5	4.9
*Phenylalanine	2.8	5.3	4.2	6.0	5.9	6.4	7.4
*Isoleucine	4.2	3.6	3.1	4.1	3.6	4.1	4.3
*Leucine	4.8	6.3	6.3	7.1	7.1	7.1	7.2
*Lysine	4.2	2.2	2.1	2.5	1.9	2.9	2.4

*Essential amino acids.

[1] Values are means of two determinations.

[2] Adapted from Cluskey et al (1976).

Wu et al (1979) showed that the nutrient quality of protein concentrates is highly dependent on barley variety. For example, protein concentrates obtained from Riso 1508 were not deficient in any of the EAA's but Hiproly and CI 4362 both had inadequate lysine, methionine and threonine values. Fellers et al (1966) showed that due to alkali extraction, proteins extracted from wheat bran decreased in protein efficiency ratio (PER) to 2.0 from 2.2 (water extracted wheat bran protein at pH 6.45). Thus, alkali extraction only slightly reduces the nutritionally quality of protein concentrates. Wu (1978) extracted protein concentrates from normal and high-lysine sorghums using alkali extraction (0.1-0.15*M* NaOH, pH 11.8-11.9) conditions and determined that even though the lysine content decreased in the protein concentrates, its bioavailability was still high as no lysinoalanine complexes were formed. In this study, the presence of lysinoalanine was not determined where all AA peaks in the HPLC traces obtained were accounted for with no unidentified peaks.

4.4. CONCLUSIONS

The solvent type and pH affect the protein concentrate in terms of the amount of protein extracted from barley, purity and percent recovery. In this study, best protein extraction results were achieved with 0.035*M* NaOH at pH 11 and 23°C for both barley varieties and their corresponding WGF, 19% PF and 19% PGF samples. Increasing the extraction temperature to 40°C did not affect protein recovery, but at 50°C, both protein purity and recovery from 19% PGF were lower for both barley varieties. Differences in protein recovery and purity values were evident within both barley types and flour fractions. This may be due to the variation in the protein type (albumins, globulins, prolamins and glutelins) and the quantities present in the grain, which will affect protein solubility under the extraction conditions used.

It appears that β-glucan recovery is more affected by variety, temperature and pH more so than the solvent type. The purity and recovery of starch is dependent on the extraction efficiency and solubility of other major components (i.e. protein and β-glucan) present within a given flour sample. Therefore, using 0.035*M* NaOH at pH 11 and by increasing the extraction temperature to 40 or 50°C resulted in a high recovery of proteins, β-glucan and starch in 19% PGF.

The nutritional quality of alkali-extracted protein concentrates diminished slightly due to decreased lysine content. In this study, protein concentrates extracted from Condor WGF, 19% PF and 19% PGF had EAA (except lysine) amounts that either met or exceeded the requirements set out by the FAO.

4.5. REFERENCES

Beer, M.U., Arrigoni, E., and Amado, R. 1996. Extraction of oat gum from oat bran: Effects of process on yield, molecular weight distribution, viscosity and (1→3)(1→4)-β-D-glucan content of the gum. Cereal Chem. 73(1): 58-62.

Beer, M.U., Wood, P.J., Weisz, J., and Fillion, N. 1997. Effect of cooking and storage on the amount and molecular weight of (1→3)(1→4)-β-D-glucan extracted from oat products by an in vitro digestion system. Cereal Chem. 74(6): 705-709.

Bhatty, R.S. 1995. Laboratory and pilot plant extraction and purification of β-glucans from hull-less barley and oat brans. J. Cereal Sci. 22: 163-170.

Chen, L., and Houston, D.F. 1970. Solublization and recovery of protein from defatted rice bran. Cereal Chem. 47(1): 73-79.

Cluskey, J.E., Wu, Y.V., Wall, J.S., and Inglett, G.E. 1973. Oat protein concentrates from a wet-milling process: Preparation. Cereal Chem. 50(4): 475-481.

Cluskey, J.E., Wu, Y.V., Inglett, G.E., and Wall, J.S. 1976. Oat protein concentrates for beverage fortification. J. Food. Sci. 41: 799-804.

Dawkins, N.L., and Nnanna, I.A. 1993. Oat gum and β-glucan extraction from oat bran and rolled oats: Temperature and pH effects. J. Food Sci. 58(3): 562-566.

Fellers, D.A., Sinkey, V., Shepherd, A.D., and Pence, J.W. 1966. Solubilization and recovery of protein from wheat millfeeds. Cereal Chem. 43(2): 1-13.

Malkki, Y., Autio, K., Hanninen, O., Myllymaki, O., Pelkonen, K., Suortii, T., and Torronen, R. 1992. Oat bran concentrates: Physical properties of β-glucan and hypocholesterolemic effects in rats. Cereal Chem. 69(6): 647-653.

Newman, R.K., and Newman, C.W. 1991. Barley as a food grain. Cereal Foods World. 36(9): 800-805.

Palmer, G.H. 1986. Levels of alkali-soluble β-glucans in cereal grains. J. Inst. Brew. 10(92): 461-462.

SAS Institute Inc. 1999. SAS/STAT *User's Guide*, version 8, SAS Institute, Cary, North Carolina.

Temelli, F. 1997. Extraction and functional properties of barley β-glucan as affected by temperature and pH. J. Food. Sci. 62(6): 1194-1197, 1201.

Wood, P.J., Siddiqui, I.R., and Paton, D. 1978. Extraction of high-viscosity gums from oats. Cereal Chem. 55(6): 1038-1049.

Wood, P.J., Weisz, J., Fedec, P., and Burrows, V.D. 1989. Large-scale preparation and properties of Oat fractions enriched in (1→ 3)(1→ 4)-β-D-glucan. Cereal Chem. 66(2): 97-103.

Wu, Y.V., and Sexson, K.R. 1975. Preparation of protein concentrate from normal and high-protein wheats. J. Agric. Food Chem. 23(5): 903-905.

Wu, Y.V., and Sexson, K.R. 1976. Protein concentrate from normal and high-lysine corns by alkaline extraction: Preparation. J. Food. Sci. 41: 509-511.

Wu, Y.V., Sexson, K.R., and Wall, J.S. 1976. Triticale protein concentrate: Preparation, composition, and properties. J. Agric. Food Chem. 24(3): 511-517.

Wu, Y.V. 1978. Protein concentrate from normal and high-lysine sorghums: Preparation, composition and properties. J. Agric. Food Chem. 26(2): 305-309.

Wu, Y.V., Sexson, K.R., and Sanderson, J.E. 1979. Barley protein concentrate from high-protein, high-lysine varieties. J. Food. Sci. 44: 1581-1583.

Wu, Yv., Stringfellow, A.C., and Inglett, G.E. 1994. Protein-and β-glucan enriched fractions from high-protein, high- β-glucan barleys by sieving and air classification. Cereal Chem. 71(3): 221-223.

Zeng, D., Doehlert, D.C., and Moore, W.R. 1998. Rheological properties of (1→3)(1→4)-β-D-glucans from raw, roasted, and steamed oat groats. Cereal Chem. 75(4): 433-438.

5. FUNCTIONAL PROPERTIES OF ALKALI-EXTRACTED BARLEY PROTEINS[1]

5.1. INTRODUCTION

The functional properties of proteins are described by their ability to contribute to the physical behavior of foods during preparation, processing and storage (Ahmedna et al 1999). Major functional properties of importance include emulsification, foam formation/stability, viscosity, gelation, fat- and water-binding ability and sensory attributes such as appearance, texture and flavor (Kinsella 1976). The utilization of a protein source depends on the desired end function that is to be achieved in the final product (Ahmedna et al 1999). The choice of a protein ingredient incorporated into foods is important, as they should meet a combination of functional, nutritional and economic goals.

Proteins' unique chemical and compositional structure (primary, secondary, tertiary and quaternary) and its ability to interact with other food components such as lipids and carbohydrates (through the formation of hydrogen bonding and hydrophobic interactions) make it a versatile functional ingredient. One such essential interaction is with water. Hydration capacity of protein concentrates is of major importance as it is used as an indicator of other functional properties such as nitrogen solubility, emulsification, viscosity and gelation (Vani and Zayas 1995). The concentration of polar groups present on protein side chains (hydrophilic regions of the polypeptide chain) are responsible for the protein-water interface (Jones and Tung 1983).

Ideally, it would be beneficial if a single protein source could satisfy all of the above mentioned functionalities. Processing conditions such as heat, pH, salts, reducing/oxidizing agents and ionic strength all affect the conformational structure of proteins, which in turn affects how they behave in food systems (Pomeranz 1991). Due to these factors, a specific protein may not possess all the desired characteristics (amino acid profile, functional and sensory attributes) to adequately perform the given tasks, thus different protein sources need to be utilized (Berardi and Cherry 1981; Phillips et al 1994).

---

[1] A version of this chapter is to be submitted to the Journal of Food Science for consideration for publication

High-protein types of foods originating from cereal grains are not very common in the market place. Proteins of wheat have gained acceptance due to their unique functionality in bread making and other bakery uses rather than their nutritional content (Kasarda 1970; Shewry et al 1994). Since the late 1980's, soybean products have dominated the North American food chain due to their nutritional content and the globular proteins exhibiting a desirable fibre-like structure similar to meat products (Kelly and Pressey 1966; Ishino and Okamoto 1975; Hermansson 1978; Garcia et al 1997). The negative attribute associated with soybean proteins is their "beany" taste, but this is overcome by masking the flavor in most foods with either sauces or other spice ingredients.

Efforts have been made to isolate protein concentrates from both oilseeds and cereal grains. The most common method has been the use of alkali extraction followed by isoelectric precipitation of the corresponding proteins. The protein concentrates have a higher protein and amino acid content as compared to the whole grain. Functionality of protein concentrates have been evaluated on oat (Cluskey et al 1973; Cluskey et al 1976; D'Appolonia and Youngs 1978; Ma 1983a; Ma 1983b; Ma and Harwalkar 1984), corn (Wu and Sexon 1976; Phillips and Sternberg 1979; Lin and Zayas 1987), wheat (Shewry et al 1994; Hettiarachchy et al 1996), soybean (Yasumatsu et al 1972; Berardi and Cherry 1981; Hayakawa and Nakai 1985) and peanuts (Rhee et al 1973) to assess their potential incorporation into food systems such as beverages, bakery, meat products, sauces and dressings. Wu et al (1979) extracted protein concentrates from whole grain flour using three high-protein and high-lysine barley varieties. Hydration capacity of protein concentrates ranged from 2.70-3.70 g water/g protein whereas the emulsion stability values were between 1 to 6%, which were considerably lower than those for soybean protein concentrates. Barley germ protein isolates exhibited high hydration capacity (400%) and high fat absorption ability (200%), which were comparable to those of both wheat and corn protein isolates (Lasztity et al 1995). However, the literature lacks information on the functional properties of protein concentrates isolated from barley whole grain flour, pearling (bran) flour and pearled grain flour. The protein content and distribution of protein classes (albumin, globulin, hordein and glutelin) may vary between the different anatomical parts of the grain, which may lead to unique functional properties of the proteins extracted from various parts of the grain. Therefore, the objectives of this study were:

1. to carry out proximate analysis of protein concentrates extracted from Candle and Condor barley whole grain flour (WGF), 19% pearling flour (19% PF) and 19% pearled grain flour (19% PGF), and
2. to determine the functional properties (hydration capacity, nitrogen solubility, emulsion stability, foaming capacity and foam stability and the extent of protein denaturation using differential scanning calorimetry) of protein concentrates extracted from Candle and Condor barley WGF, 19% PF and 19% PGF.

5.2. MATERIALS AND METHODS

5.2.1. Materials

Candle and Condor barley grains were obtained and pearled to 19% level as described in Chapter 3. Alkali extraction of Candle and Condor barley whole grain flour protein concentrates (WGFpc) and 19% pearling flour protein concentrates (19% PFpc) were carried out at 23°C, whereas 19% pearled grain flour protein concentrates (19% PGFpc) were extracted at 23, 40 and 50°C using NaOH. Protein extraction was carried out according to the protocol highlighted in Chapter 4. Proximate analysis of the protein concentrates was performed according to standard methodologies given in Chapter 3.

5.2.2. Functional properties

5.2.2.1. Differential scanning calorimetry

A Differential Scanning Calorimeter (DSC) (Model 910) fitted with a TA Instruments DSC cell (Model 19720-0311, Du Pont Instruments, New Castle, DE) was used. Samples (3-8 mg of dry material and 5-20 mg of distilled water) were placed in aluminum DSC pans and hermetically sealed. The DSC cells were allowed to equilibrate for approximately 45 min at room temperature (23°C) prior to analysis. An empty DSC pan was used as blank against the samples. Sample and blank reference pans were heated from 30°C to 130°C at a rate of 10°C/min (Lupano and Gonzalez 1999). Triplicate DSC curves were obtained for each of the protein concentrate samples.

5.2.2.2. Hydration capacity

Modified procedures of Ahmedna et al (1999) and Lin and Zayas (1987) were used to determine the hydration (water holding) capacity of the protein concentrates. Samples (1%, w/v, protein in water) were dispersed in 50 mL distilled water. The pH was adjusted to 7.0 with either NaOH or HCl at room temperature (23°C). After pH adjustment, the samples were centrifuged at 1,200 x *g* for 15 min. Hydration capacity was calculated as the difference between the wet (hydrated) weight and original dry weight and expressed in grams of water absorbed per gram of protein. Analyses were done in duplicate for each sample.

5.2.2.3. Nitrogen solubility

Protein solubility was determined using modified procedures of Hettiarachchy et al (1996) and Wu et al (1979). Samples (1%, w/v, protein in water) were dispersed in 50 mL distilled water. The pH was adjusted to 7.0 with either NaOH or HCl. The solutions were mixed at room temperature (23°C) using a magnetic stirrer for 30 min with pH adjustment to 7.0 as needed. The samples were then centrifuged at 2,750 x *g* for 25 min. The supernatant was decanted and filtered using a Whatman# 4 filter paper. The undissolved protein residue was collected by scraping and washing the filter paper with distilled water. Both the supernatant and the protein residue were freeze-dried. Nitrogen content of the freeze-dried samples was determined using a nitrogen analyzer (Model FP-428, Leco Instruments Ltd., Mississauga, ON) and the values were converted to percent protein using a conversion factor of 6.25. Protein solubility in the supernatant was calculated as the percentage of total protein in the starting samples. Duplicate runs were carried out for each sample.

5.2.2.4. Emulsion stability

For the determination of emulsion stability, modified procedures of Hettiarachchy et al (1996), Yasumatsu et al (1972) and Ahmedna et al (1999) were used. Samples (1%, w/v, protein in water) were dispersed in 50 mL distilled water. The pH was adjusted to 7.0

with either NaOH or HCl. The solutions were mixed at room temperature (23°C) for 30 min using a magnetic stirrer with pH adjustment to 7.0 as needed. Then, 50 mL canola oil was added (total volume 100 mL) and the mixture was homogenized for 2 min (16,200 rpm, Polytron homogenizer, Brinkmann Instruments, Rexdale, ON). The emulsions were transferred to two 50 mL graduated centrifuge tubes and the amount of emulsion formed (emulsion activity) was determined by measuring the volume of emulsion expressed as a percentage of the total volume occupied in the tubes. The mixtures were allowed to sit at room temperature for 1 hr. The emulsion samples were then heated to 80°C in a water bath and held for 30 min. The mixtures were cooled (cold-water bath) to 23-25°C and then centrifuged (IEC-Centra, NT4 International Equipment Co., Needham Heights, MA) at 2,750 x *g* for 15 min. Emulsion stability at 23°C, 80°C and after centrifugation (2,750 x *g*) was calculated by measuring the volume of water and oil separation as a percentage of emulsion activity. Duplicate runs were carried out for each sample.

5.2.2.5. Foaming capacity and stability

Samples (1%, w/v, protein in water) were dispersed in 100 mL distilled water. The pH was adjusted to 7.0 with either NaOH or HCl. The solutions were mixed at room temperature (23°C) using a magnetic stirrer for 30 min with pH adjustment to 7.0 as needed. The samples were then transferred to a glass bowl with straight and smooth edges. The samples were mixed for 2 min using a hand-held mixer (Sunbeam, with two mixing prongs, set at high mixing speed) and then transferred to either 250 or 500 mL graduated cylinders and covered. Percent foaming capacity was calculated as the volume of foam generated per 100 mL of starting liquid (Ahmedna et al 1999). The samples were then allowed to sit for 30 and 60 min at room temperature and foaming stability was measured as the volume of foam remaining after each time interval as a percentage of the original foam volume (Temelli 1997). Duplicate runs were carried out for each sample.

5.2.3. Statistical analysis

Compositional analyses results are averages of duplicate determinations on each sample. Analysis of variance of results was done using General Linear Model (GLM) procedure of SAS Statistical Software, Version 8 (SAS Institute 1999). The model consisted of the main effects of flour type, temperature and flour type*temperature interaction effect on protein water holding capacity, nitrogen solubility, emulsion stability, foaming capacity and foam stability. Multiple comparison of means was performed by Tukey's studentized range (HSD) test at $\alpha=0.05$ level.

5.3. RESULTS AND DISCUSSION

5.3.1. Proximate analysis

Table 5.1 highlights the proximate analysis of WGFpc, 19% PFpc and 19% PGFpc for Candle barley. Protein contents of WGFpc and 19% PGFpc were similar 80.3% and 82.3%, respectively. Protein content was lower (70.2%) in 19% PFpc, but this fraction also had the highest fat content of 15.2%. The fat content in WGFpc was 9.7% with the lowest value being in the 19% PGFpc (5.6%), as expected. Starch content in WGFpc and 19% PFpc was 0.8% whereas in 19% PGFpc it was 1.4%. β-Glucan content in WGFpc and 19% PGFpc was in the range of 1.1-1.5%, but in 19% PFpc, it was only 0.5%.

Increasing the protein extraction temperature of Candle 19% PGF to 40°C (Table 5.2) resulted in an increase in the level of moisture, starch, β-glucan and ash contents compared to those obtained at 23°C (Table 5.1). Protein content dropped by 5.9% to

Table 5.1. Proximate analysis of Candle and Condor barley protein concentrates extracted from WGF, 19% PF and 19% PGF using 0.035*M* NaOH at pH 11 and 23°C.

Analysis	**% (w/w, dry matter basis)**					
	Candle			**Condor**		
	WGFpc	**19% PFpc**	**19% PGFpc**	**WGFpc**	**19% PFpc**	**19% PGFpc**
Moisture	3.0±0.2	2.9±0.1	4.4±0.2	3.7±0.4	3.7±0.2	4.7±0.8
Starch	0.8±0.0	0.8±0.1	1.4±0.0	0.3±0.0	0.2±0.0	0.3±0.0
Protein	80.3±0.6	70.2±0.1	82.3±0.4	87.8±0.6	75.0±0.8	92.0±0.2
β-Glucan	1.1±0.1	0.45±0.0	1.5±0.0	0.4±0.0	0.2±0.0	0.4±0.0
Fat	9.7±0.0	15.2±2.2	5.6±0.4	3.1±0.2	10.2±0.9	1.4±0.0
Ash	2.0±0.1	2.2±0.0	2.2±0.2	1.6±0.0	2.2±0.1	1.4±0.0

Table 5.2. Proximate analysis of Candle and Condor barley protein concentrates extracted from 19% PGF using 0.035*M* NaOH at pH 11 and 40 and 50°C.

Analysis	**% (w/w, dry matter basis)**			
	Candle 19% PGFpc		**Condor 19% PGFpc**	
	40°C	**50°C**	**40°C**	**50°C**
Moisture	6.3±0.2	7.9±0.2	6.3±0.1	4.6±0.0
Starch	2.6±0.1	2.4±0.4	0.3±0.0	0.4±0.1
Protein	76.4±0.4	78.3±0.4	86.6±0.2	87.7±0.8
β-glucan	2.0±0.2	1.8±0.3	0.8±0.0	1.2±0.0
Fat	0.8±0.0	4.7±0.0	0.4±0.0	1.0±0.1
Ash	3.1±0.1	2.5±0.2	1.8±0.1	2.5±0.0

76.4% and the fat content also decreased from 5.6% to 0.8% with an increase in extraction temperature from 23°C to 40°C. A further increase in protein extraction temperature to 50°C caused an increase in protein content from 76.4% (40°C) to 78.3% but the value was still below 82.3%, which was obtained at 23°C extraction. Increasing the extraction temperature from 40°C to 50°C resulted in minimal changes in starch and β-glucan contents, but the ash content dropped to 2.5%, whereas the fat content increased to 4.7%.

For Condor barley, the protein content of 19% PGFpc was highest at 92% followed by WGFpc (87.8%), while the lowest protein concentration was obtained in 19%

PFpc (75.0%) (Table 5.1). The protein contents of Condor barley protein concentrates were higher as compared to those of Candle, but the trend in the protein content of the concentrates obtained from the different flour fractions was similar in both varieties. Concentrations of both starch and β-glucan were low with values being less than 0.5% as compared to Candle. The fat contents of Condor barley protein concentrates were lower than those of Candle. But, as with Candle barley, the fat content in Condor 19% PFpc was highest with a value of 10.2%, followed by WGFpc (3.1%) and lowest in 19% PGFpc where the fat content was only 1.4%. Ash content was highest in 19% PFpc (2.22%) whereas in WGFpc and 19% PGFpc it was 1.4% and 1.6%, respectively.

As compared to room temperature (23°C) extraction (Table 5.1), the moisture content (Condor 19% PGFpc) increased to 6.3% at an extraction temperature of 40°C but then dropped down to 4.6% at 50°C for 19% PGFpc. (Table 5.2). The protein content of 19% PGFpc at 40°C and 50°C remained in the range of 86.6-87.7%, but this was lower than that obtained at 23°C (92.0%). Increasing the protein extraction temperature did not affect the starch content of Condor 19% PGFpc, but both β-glucan and ash contents increased with extraction temperature. Fat content of Condor 19% PGFpc decreased to 0.4% when extracted at 40°C but then increased to 1.0% at an extraction temperature of 50°C. This trend was also observed in Candle 19% PGFpc.

A direct method to determine the fat binding capacity was not done on the protein concentrates of Candle and Condor barley. But indirectly, it can be observed that Candle protein concentrates from WGF, 19% PF and 19% PGF have a higher affinity to bind fat as compared to those of Condor (Table 5.1). Based upon the fat content of the starting material of individual flour fractions (Table 3.1 and 3.2 in Chapter 3), Candle barley

WGFpc and 19% PGFpc contain 40-41% of the total fat in the starting whole grain flour whereas the value for 19% PFpc is 34%. For Condor, WGFpc retained 19% of the total fat in the starting flour with 19% PGFpc binding 13% and 19% PFpc binding 28% of the total fat. One possible explanation for these observations could be due to the fact that alkaline extraction (pH 11) of proteins using NaOH may have caused the saponification of barley triglycerides to form free fatty acids. Through amide bonding (between amine group of protein and carboxyl group of fatty acid), proteins may bind fatty acids to form lipoproteins (Morrison and Boyd 1987). Thus, when the pH of the extract solution is brought down to the isoelectric point of the proteins (pH 5.5) during the extraction process, the bound fatty acids will also precipitate out along with the proteins.

5.3.2. Functional properties

5.3.2.1. Differential scanning calorimetry

Figures 5.1 and 5.2 show the DSC graphs of Candle and Condor barley starting flour fractions, respectively. Only single endothermic peaks are present, which is due to the gelatinization of starch. Additional peaks representing protein denaturation were not seen due to perhaps a low content of protein, due to the resistance of barley proteins to heat denaturation in the temperature range studied and/or due to the protein denaturation peak possibly being hidden under the starch gelatinization peak. In a mixture, containing 48% whey protein concentrate and 97% cassava starch, two endothermic peaks were obtained due to starch gelatinization (72°C) and protein denaturation (91°C) by Lupano and Gonzalez (1999). Oat 46% protein concentrate (obtained by salting out with NaCl and dialysis and subsequent spray drying) had a single endothermic peak at 112°C, indicating a strong and highly interactive protein network (Arntfield and Murray 1981). Starch content

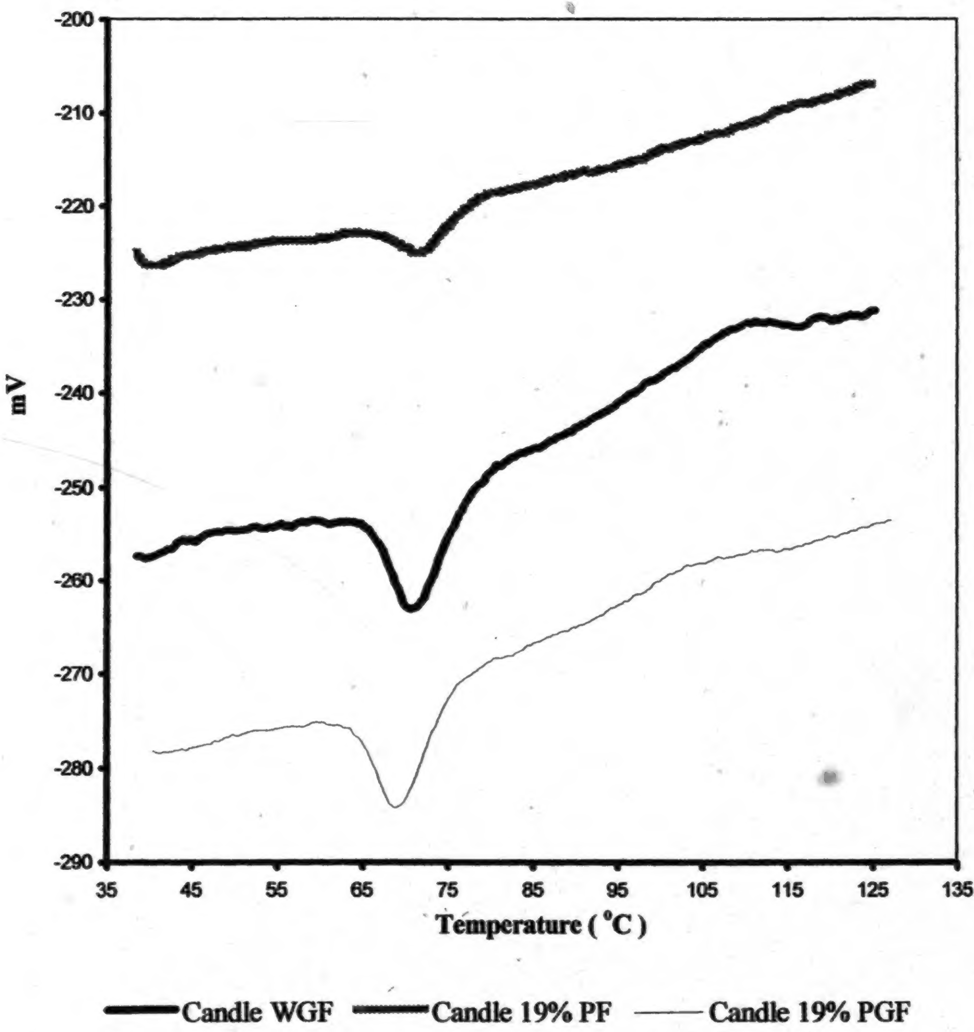

Figure 5.1. DSC results for Candle barley WGF and pearling fractions.

of Candle and Condor barley 19% PGF was the highest, ranging from 70-72% followed by WGF (59-61%) and lowest in 19% PF (18-19%) (Table 4.1 in Chapter 4), whereas the protein content of Candle and Condor barley 19% PF was the highest, ranging from 25-

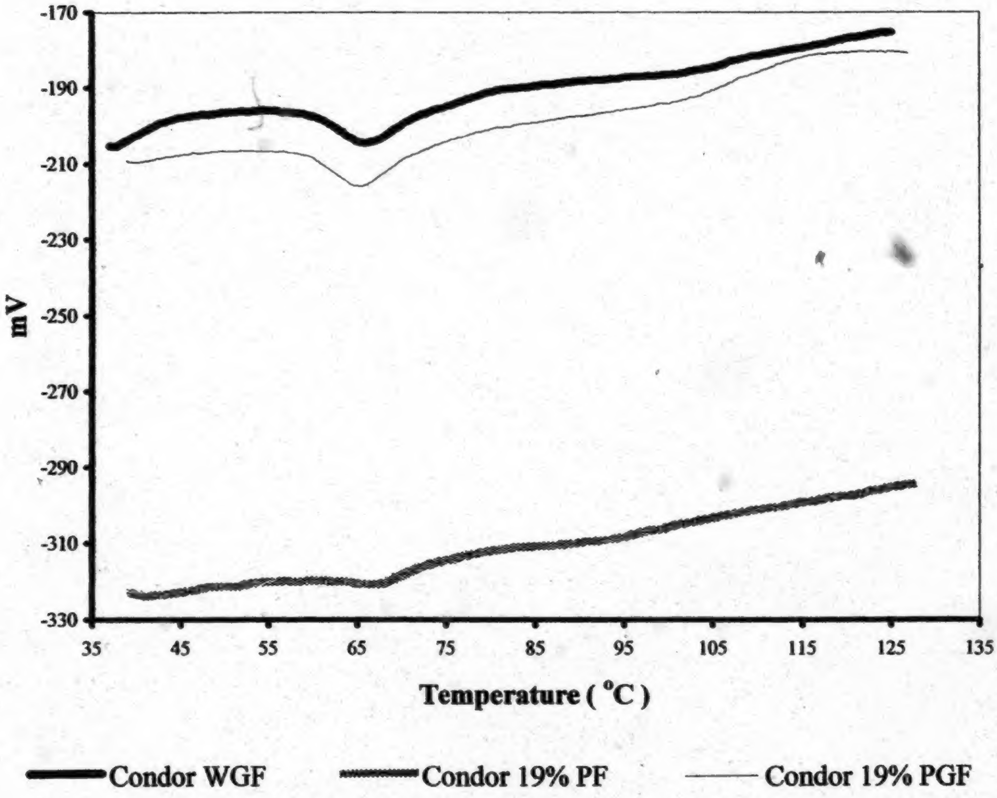

Figure 5.2. DSC results for Condor barley WGF and pearling fractions.

29% followed by WGF (14-18%) and lowest in 19% PGF (12-16%) (Table 4.1 in Chapter 4). Differences in the endothermic peak shapes between the flour fractions of the two barley varieties (Figs. 5.1 and 5.2) are due to the differences in starch type and their starch and protein contents. In general, enthalpy (ΔH, area under the DSC curve) and peak temperature (Tp) are higher for waxy (Candle barley) than normal (Condor barley) starches, as demonstrated by Li et al (2000) and the results of this study.

Figures 5.3 and 5.4 show the DSC graphs of the protein concentrates extracted from WGF, 19% PF and 19% PGF of Candle and Condor barley, respectively. No endothermic peaks can be observed on these graphs indicating that the protein concentrates have been completely denatured and the residual starch is too small to be observed. Severe alkaline (pH 10-14) conditions cause ionization of the acidic side chains present on a polypeptide, creating negative charges (mainly on the carboxyl group). The charged polypeptides repel each other while still attracting water molecules, which result in the disruption of the hydrophobic regions within the protein molecule, causing it to unfold (Kinsella 1976). Arntfield and Murray (1981) also showed that there were no endothermic peaks for a commercial soy isolate that underwent alkaline treatment whereas soy protein isolate extracted with NaCl at neutral pH (6.2) showed denaturation (endothermic peak) at 91°C.

If proteins are not completely denatured, they can re-aggregate into a stabilized structure if the pH is adjusted back to the isoelectric point. Transition temperature of protein denaturation of whey protein increased from 91°C (pH 3.75) to 93°C at pH 4.2 (Lupano and Gonzalez 1999). The protein concentrates from Candle and Condor barley were neutralized (pH 6.6-7.2) prior to DSC analysis. By neutralizing the protein concentrate solutions, the pH is brought closer to the isoelectric point (pH 5.5) which may have caused reassociation of the polypeptide chains, but this was not evident in Figures 5.3 and 5.4. Fababean proteins that were extracted at pH 12 and acidified at pH 4.5 (isoelectric point) (with no pH adjustment before DSC analysis) showed no endothermic peak but proteins extracted at pH 8 exhibited a peak at 82°C (Arntfield and Murray 1981). If a protein concentrate or isolate is only partially denatured, the area under the curve will

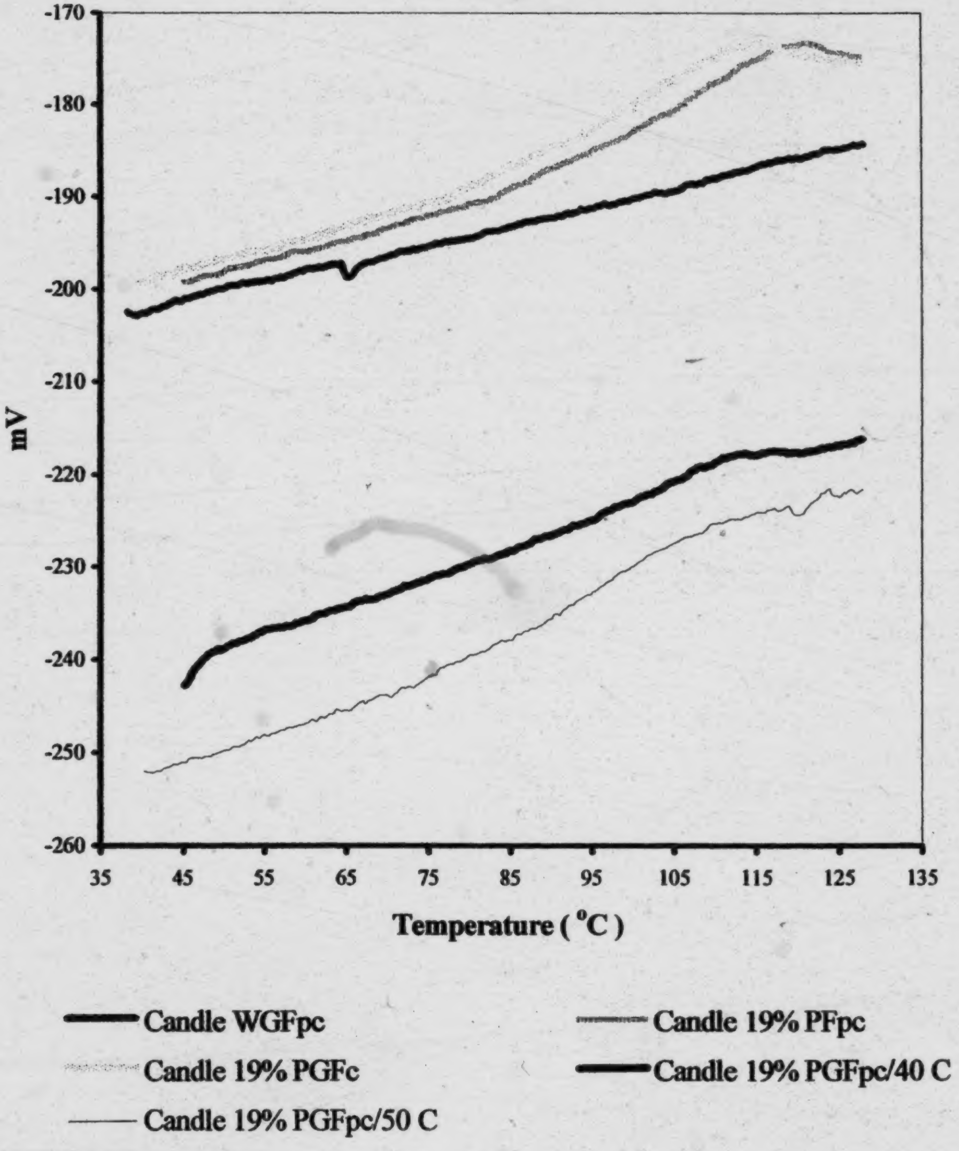

Figure 5.3. DSC results for Candle barley protein concentrates obtained from WGF and pearling fractions at various temperatures.

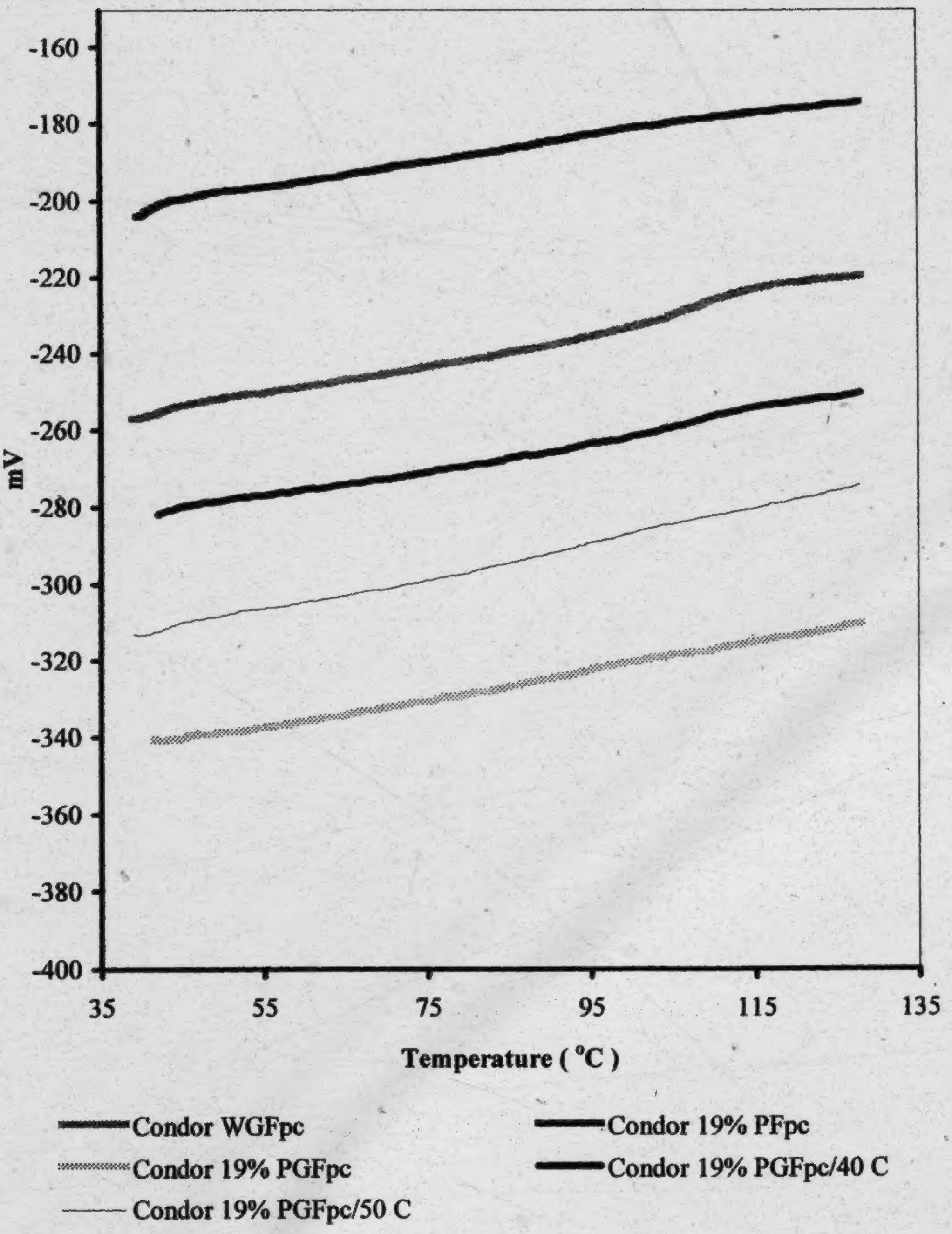

Figure 5.4. DSC results for Condor barley protein concentrates obtained from WGF and pearling fractions at various temperatures.

decrease, but if the protein source is entirely denatured, then no peaks will appear upon DSC analysis (Biliaderis 1983).

DSC analysis of vital gluten (77% protein content) resulted in two major exothermic peaks, one at 80°C and the other at 117°C, which would indicate reassociation of hydrophobic regions and polar interactions of polypeptide chains (Arntfield and Murray 1981). In Figure 5.3, Candle barley 19% PFpc and 19% PGFpc showed exothermic peaks between 110 and 117°C. The DSC analysis was stopped at 120°C and thus the end points of the curves (conclusion temperature) could not be determined to evaluate the completeness of the curve. More detailed experiments are needed to confirm these findings. No such peaks were obtained for Condor barley protein concentrates either (Fig. 5.4).

5.3.2.2. Hydration capacity

Hydration capacity (Table 5.3) of Candle WGFpc and 19% PFpc were 5.4 and 5.7, respectively. Increasing the protein extraction temperature to 40°C and 50°C significantly ($P \leq 0.05$) increased the water holding capacity of 19% PGFpc from 4.3 (at 23°C) to 7.5-7.6. The hydration capacities of all Condor barley protein concentrates (Table 5.3) were similar ($P > 0.05$) and ranged from 4.5 to 5.1. There was no effect ($P > 0.05$) of extraction temperature on the hydration capacity of 19% PGFpc.

Vani and Zayas (1995) obtained highest water retention values (1.9 g water/g protein) for wheat germ protein isolates extracted at pH 8 and 70°C. Ma (1983a,b) determined water holding capacity of several alkali extracted oat protein concentrates to be 0.8 mL water/g protein. Wu and Sexon (1976) found hydration capacity of corn protein isolates to be between 3.5 to 4.2 g water/g protein, whereas water absorption values of

Table 5.3. Hydration capacity (g H_2O/g protein) and nitrogen solubility of protein concentrates extracted from Candle and Condor barley WGF, 19% PF and 19% PGF using 0.035*M* NaOH at pH 11 and 23, 40 and 50°C.

Variety	Protein concentrate	Water holding capacity	% Nitrogen solubility
Candle	WGFpc	5.4^{bc}	16.6^{bc}
	19% PFpc	5.7^{b}	13.3^{c}
	19% PGFpc	4.3^{c}	21.3^{bc}
	19% PGFpc/40°C	7.5^{a}	26.1^{b}
	19% PGFpc/50°C	7.6^{a}	43.3^{a}
Condor	WGFpc	5.0^{a}	12.6^{c}
	19% PFpc	5.1^{a}	14.7^{c}
	19% PGFpc	4.5^{a}	17.2^{c}
	19% PGFpc/40°C	5.1^{a}	26.2^{b}
	19% PGFpc/50°C	4.9^{a}	41.1^{a}

$^{a-c}$Means with different letters for a given variety within a column are significantly different ($P \leq 0.05$).

soybean, cottonseed and peanut protein isolates (alkaline extraction using NaOH at pH 10.5 with isoelectric point precipitation at pH 5) were in the range of 2.8-3.4 mL water/ g protein (Berardi and Cherry 1981). Candle and Condor barley protein concentrates have higher hydration capacities (4.3-7.6 g H_2O/ g protein, Table 5.3) as compared to other sources of cereals and oilseeds, thus they can be expected to provide better hydration function in a variety of food products such as meat products where cooking loss due to water separation is not a desired characteristic.

5.3.2.3. Nitrogen solubility

For Candle, nitrogen solubility was lower in 19% PFpc (13.3%) as compared to WGFpc (16.6%) and 19% PGFpc (21.3%) whereas for Condor, nitrogen solubility of WGFpc, 19% PFpc and 19% PGFpc was 12.6%, 14.7% and 17.2%, respectively. There was no significant difference ($P > 0.05$) in the nitrogen solubility of protein concentrates extracted from the different flours of each barley type. Increasing the protein extraction temperature from 23°C to 40°C did increase ($P \leq 0.05$ for Condor, but did not reach statistical significance in Candle) nitrogen solubility in 19% PGFpc for both varieties (26%) but the values were significantly ($P \leq 0.05$) higher (41-43%) for 19% PGFpc/50°C. No direct correlation could be established between hydration capacity and nitrogen solubility.

Wu et al (1979) showed that at pH levels close to the isoelectric point of barley proteins (pH 4.5-5.5), nitrogen solubility was lowest due to protein aggregation into a stable structure. However, pH values on either side of the isoelectric point increased nitrogen solubility, even though alkaline pH affects nitrogen solubility to a greater extent as compared to acid pH range. Highest solubility at the acid pH range (pH 1-1.5) resulted in only 50-60% nitrogen solubility as compared to 80-90% at pH 9-10. At neutral pH (6-7), nitrogen solubility ranged from 18-20% (23-25°C extraction) (Wu et al 1979), which is consistent with the results obtained in this study.

5.3.2.4. Emulsion stability

The ability of Candle and Condor barley protein concentrates to form stable emulsions at various time/temperature conditions is presented in Table 5.4. The original emulsion activity for both Candle and Condor barley protein concentrates was 100% (i.e. ability to form an emulsion under the specified conditions). For Candle barley, emulsion stability decreased to 97.5% for 19% PFpc but remained at 100% for the other protein fractions after 1 hr at 23°C. For Condor barley, emulsion stability decreased to 95.0% for both 19% PFpc and 19% PGFpc/40°C but the largest drop was seen in WGFpc where the stability was only 90%.

The effect of high temperature (80°C) on emulsion stability had mixed results for both Candle and Condor barley protein concentrates. In Candle barley, emulsion stability

Table 5.4. Emulsion stability of protein concentrates extracted from Candle and Condor barley WGF, 19% PF and 19% PGF using 0.035*M* NaOH at pH 11 and 23, 40 and 50°C.

Variety	**Protein concentrate**	**% Emulsion stability (23°C/1 hr)**	**% Emulsion stability (80°C/30 min)**	**% Emulsion stability (3250 x g/15 min)**
Candle	WGFpc	100.0	100.0	56.2^{ab}
	19% PFpc	97.5	77.5	52.5^{b}
	19% PGFpc	100.0	95.0	57.5^{ab}
	19% PGFpc/40°C	100.0	100.0	58.1^{ab}
	19% PGFpc/50°C	100.0	96.0	62.5^{a}
Condor	WGFpc	90.0	81.9	54.3^{a}
	19% PFpc	95.0	93.8	55.0^{a}
	19% PGFpc	100.0	87.5	55.0^{a}
	19% PGFpc/40°C	95.0	90.0	57.5^{a}
	19% PGFpc/50°C	100.0	96.0	61.2^{a}

$^{a-b}$Means with different letters for a given variety within a column are significantly different ($P \leq 0.05$).

for 19% PFpc fell to 77.5% but remained between 96-100% for the other four protein concentrates. For Condor barley, emulsion stability was lowest for WGFpc (81.9%) but for the other protein concentrates, emulsion stability remained between 87.5-96%. After centrifugation, emulsion stability dropped to 52.5-62.5% for both Candle and Condor barley protein concentrates. The emulsion stability after centrifugation was significantly ($P \leq 0.05$) lower for Candle barley 19% PFpc (52.5%) but there was no significant ($P < 0.05$) difference between the Condor barley protein concentrates.

Hettiarachchy et al (1996) obtained emulsion stability of 61.2% (held at 70°C for 1 hr) for wheat germ protein isolate. Wu et al (1976) determined emulsion stability (held at 70°C for 30 min) of several varieties of alkali extracted triticale protein concentrates to be in the range of 81-87%, whereas for commercial soybean protein isolate, the value was only 45%. Emulsion stability (held for 30 min at 70°C) values, for high-lysine and normal corn varieties, were 21 to 54% (Wu and Sexson 1976) but for Garland oat protein concentrate, emulsion stability (held for 30 min at 70°C) was between 56-57% (Wu and Cluskey 1973). Emulsion stability (held at 80°C for 30 min) values obtained in this study for both Candle and Condor barley protein concentrates either met or exceeded the values reported for other cereal grains.

The ability of a protein to stabilize an emulsion is based upon its denaturation characteristics. Disruption of the hydrophobic regions of the protein molecule and exposure of the polar groups on the polypeptide chain are responsible for creating protein-lipid and protein-water interactions at the oil-water interface. In this study, the results obtained for emulsion stability are consistent with the linear DSC curves (unfolding of proteins) for both Candle and Condor protein concentrates.

Both Candle and Condor barley protein concentrate fractions exhibited excellent emulsion activity and stability after various time, temperature and centrifugation treatments. The stable emulsions retained their flow behavior at both 23°C and 80°C. After centrifugation, the emulsions became thicker and solidified in the test tubes, where the consistency of the emulsions resembled that of mayonnaise and would not flow even after the test tubes were inverted. Further rheological tests need to be performed to evaluate the strength of the emulsions and their resistance to shear force. This will determine their suitability as a stabilizer for various food applications such as creamy salad dressings and ice cream products. Another food product that the barley protein concentrates from WGF, 19% PF and 19% PGF can be utilized in are processed meat products. Besides having the ability to form emulsions, barley protein concentrates also possess a high affinity to bind water (Table 5.3), thus they have the capacity to retain moisture and cooking flavor, while maintaining the dimensional stability of the cooked meat products (Voutsinas et al 1983).

5.3.2.5. Foaming capacity and stability

Foaming capacity and stability results are presented in Table 5.5. Candle barley WGFpc and 19% PFpc had the lowest ($P \leq 0.05$) foaming capacity (36-46%) and foam stability (10-13%), while 19% PGFpc/40°C had the highest ($P \leq 0.05$) foaming capacity at 430% and after 60 min the foam was still 72.7% stable. Candle PGFpc (extracted at 23°C) did have a high foaming capacity of 112% but with poor foam stability (8.0% after 60 min). Also, Candle 19% PGFpc/50°C showed a good ability to form a foam (200%) but it was quite unstable; stability dropped down to 17.6% after 30 min and then to 13.1% after 60 min.

Condor barley 19% PFpc had the lowest ($P \leq 0.05$) foaming capacity (47.5%) and a low stability (56.8%). Candle WGFpc had a foaming capacity of 98.5% and maintained its stability (70.7%) even after 60 min. Protein concentrates obtained from Condor barley 19% PGF all exhibited high foaming capacity and stability. Condor 19% PGFpc, 19% PGFpc/40°C and 19% PGFpc/50°C had foaming capacities of 258, 345 and 437% and foam stability after 60 min was 75.4, 85.5 and 88.0%, respectively. Overall, protein concentrates from Condor barley flour fractions (WGF, 19% PF and 19% PGF) had higher foaming capacity and stability as compared to Candle. Extraction temperature (23°C, 40°C and 50°C) of 19% PGFpc did have a significant ($P \leq 0.05$) effect on foaming capacity but not on stability for Condor barley. Even though the extraction temperature also had a significant effect on the foaming capacity and stability of Candle protein extracts, the trends were not consistent. The lack of the foam stability of Candle protein fractions could be due to the intermolecular protein-protein interactions, which may have caused the weakening of the bubble surface film resulting in breakage (Cherry and

Table 5.5. Foaming capacity and foaming stability of protein concentrates extracted from Candle and Condor barley WGF, 19% PF and 19% PGF using 0.035*M* NaOH at pH 11 and 23, 40 and 50°C.

Variety	Protein concentrate	% Foaming capacity (23°C)	% Foam stability (23°C/30 min)	% Foam stability (23°C/60 min)
Candle	WGFpc	46.0^{d}	15.2	10.9^{b}
	19% PFpc	36.5^{d}	24.6	13.8^{b}
	19% PGFpc	112.0^{c}	11.5	8.0^{b}
	19% PGFpc/40°C	430.0^{a}	86.0	72.7^{a}
	19% PGFpc/50°C	200.0^{b}	17.6	13.1^{b}
Condor	WGFpc	98.5^{d}	86.7	70.7^{ab}
	19% PFpc	47.5^{e}	58.9	56.8^{b}
	19% PGFpc	228.0^{c}	82.5	75.4^{ab}
	19% PGFpc/40°C	345.0^{b}	87.7	85.5^{a}
	19% PGFpc/50°C	437.5^{a}	89.1	88.0^{a}

$^{a-e}$Means with different letters for a given variety within a column are significantly different ($P \leq 0.05$).

McWatters 1981). The high lipid content of the Candle protein concentrates may have also influenced their foam forming and stabilizing function.

Ahmenda et al (1999) determined the foaming capacity of wheat protein isolate to be 175% with a stability of only 12% after 30 min at room temperature. The authors attributed the high foam capacity of wheat protein isolate to its protein (nitrogen) solubility, which was approximately 50%. Foaming capacity of soybean protein isolate, sodium caseinate, dry whole milk protein and dried egg white protein was within the range of 125-250% with foam stability ranging from 10-20% after 30 min at room temperature (Ahmenda et al 1999). In this study, protein concentrates with higher nitrogen solubility did in general have higher foaming capacity, but a strong direct correlation could not be established. As well, no relationship could be established regarding nitrogen solubility and foam stability of Candle and Condor protein concentrates.

Formation of foam is dependent on the proteins' ability to disperse in a given solvent, thus forming a monolayer of film at the solvent-air interface. Proteins that are in their denatured state have the ability to decrease the solvent-air interfacial tension and upon agitation (aeration), the proteins are able to entrap air into bubbles creating a foam. Furthermore, to stabilize the foam, proteins solubilize in the liquid layer surrounding the air bubble and keep it from coalescing (Cherry and McWatters 1981; Garcia et al 1997). Ma and Harwalkar (1984) extracted individual protein fractions from whole oat groats and found that they all affected foam formation uniquely. Foaming capacity of the albumin protein fraction was the highest (240%), followed by globulins (100%) and prolamin and glutelin protein fractions had the lowest foaming capacity of 45-50%. These findings of Ma and Harwalkar (1984) could not be corroborated in this study using barley proteins. In Table 3.5 (Chapter 3), it can be seen that 19% PGF of Candle and Condor barley had the lowest amounts of albumins (9-10%) and globulins (3-6%) and higher amounts of hordeins (prolamins) (42-45%) and glutelins (22-23%) even though they exhibited very high foaming capacity (112.0-437.5%) Berardi and Cherry (1981) determined the whippability of soybean, cottonseed and peanut protein isolates to be between 440-452% with foam stability ranging from 80-92% (60 min at room temperature). Alkaline extracted Hinoat and Sentinel oat protein concentrates had foaming capacities of 76-80% with foam stability of 45-50%. Candle and Condor barley 19% PGFpc extracted at 23, 40 and 50°C, all had foaming capacities above 100% with the highest level reached at 437%. Foam stability was lower for Candle protein concentrates, but for Condor, it ranged from 75-88%; therefore, alkali extracted barley proteins from 19% PGF can be used in food systems where foam formation and stabilization are needed.

5.4. CONCLUSIONS

Alkali extracted protein concentrates from Candle and Condor barley WGF, 19% PF and 19% PGF had high protein and fat contents with low β-glucan and starch contents. Increasing the protein extraction temperature of 19% PGF to 40-50°C from 23°C, decreased protein purity by 5-7%. Candle protein concentrate fractions appear to have a better ability to bind fat as compared to those of Condor.

Both Candle and Condor barley protein fractions displayed a high tendency to bind water but had somewhat low protein solubility at neutral pH. Protein solubility increased with an increase in protein extraction temperature of PGF. Protein concentrates from Candle and Condor barley would make excellent surfactants due to their ability to bind fat and form stable emulsions in an oil-water system. However, not all protein fractions were able to stabilize foams. In general, 19% PGFpc fractions from Condor barley exhibited high foaming capacity and stability. From the DSC curves, it is apparent that alkaline extraction conditions (0.035*M* NaOH and pH 11) have denatured the proteins from their quaternary form to their secondary or primary structure, such that additional heat did not have any effect on the physical nature of the proteins. Denaturation of proteins should not be considered a negative characteristic, as some degree of protein unfolding is needed to establish functionality.

5.5 REFERENCES

Ahmenda, M., P., Prinyawiwatkul, W., and Rao, M. 1999. Solubilized wheat protein isolate: Functional properties and food applications. J. Agric. Food Chem. 47: 1340-1345.

Arntfield, S.D., and Murray, E.D. 1981. The influence of processing parameters on food protein functionality. I. Differential scanning calorimetry as an indicator of protein denaturation. Can. Inst. Food Sci. Technol. J. 14(4): 289-294.

Berardi, L.C., and Cherry, J.P. 1981. Functional properties of co-precipitated protein isolates from cottonseed, soybean and peanut flours. Can. Inst. Food Sci. Technol. J. 14(4): 283-288.

Biliaderis, C.G. 1983. Differential scanning calorimetry in food research-A review. Food Chem. 10: 239-265.

Cherry, J.P. and McWatters, K.H. 1981. Whippability and aeration. Ch. 8 in *Protein Functionality in Foods*, J.P. Cherry (Ed.), p. 149-176. American Chemical Society., Washington, D.C.

Cluskey, J.E., Wu, Y.V., Wall, J.S., and Inglett, G.E. 1973. Oat protein concentrates from a wet milling process: Preparation. Cereal Chem. 50: 475-481.

Cluskey, J.E., Wu, Y.V., Inglett, G.E., and Wall, J.S. 1976. Oat protein concentrates for beverage fortification. J. Food. Sci. 41: 799-804.

D'Appolonia, B.L., and Youngs, V.L. 1978. Effects of bran and high protein concentrate from oats on dough properties and bread quality. Cereal Chem. 55(5): 736-743.

Garcia, M.C., Torre, M., Marina, M.L., and Laborda, F. 1997. Composition and characterization of soybean and related products. Crit. Rev. Food. Sci. Nutr. 37(4): 361-391.

Hayakawa, S., and Nakai, S. 1985. Relationships of hydrophobicity and net charge to the solubility of milk and soy proteins. J. Food Sci. 50: 486-491.

Hermansson, A.M. 1978: Physico-chemical aspects of soy proteins structure formation. Journal of Texture Studies. 9: 33-58.

Hettiarachchy, N.S., Griffin, V.K., and Gnanasambandan, R. 1996. Preparation and functional properties of a protein isolate from defatted wheat germ. Cereal Chem. 73(3): 363-367.

Ishino, K., and Okamoto, S. 1975. Molecular interaction in alkali denatured soybean proteins. Cereal Chem. 52: 9-20.

Jones, L.J., and Tung, M.A. 1983. Functional properties of modified oilseed protein concentrates and isolates. Can. Inst. Food Sci. Technol. J. 16(1): 57-62.

Kasarda, D.D. 1970. The conformational structure of wheat proteins. The Bakers Digest. 12: 20-26.

Kelley, J.J., and Pressey, R. 1966. Studies with soybean protein and fibre formation. Cereal Chem. (43): 195-206.

Kinsella, J.E. 1976. Functional properties of proteins in foods: A survey. Crit. Rev. Food. Sci. Nutr. 16(5): 219-280.

Lasztity, R., Tomoskozi, S., Nagy, J., and Bajkai, T. 1995. Investigation of some functional and nutritive properties of cereal germ proteins. Periodica Polytechnica Ser. Chem. Eng. 39(1): 63-76.

Li, J.H., Vasanthan, T., and Rossnagel, R.H. 2000. Starch from hull-less barley: II. Thermal, rheological and acid hydrolysis characteristics. Food Chem. 74: 407-415.

Lin, C.S., and Zayas, J.F. 1987. Functionality of defattd corn germ proteins in a model system: Fat binding capacity and water retention. J. Food Sci. 52(5): 1308-1311.

Lupano, E.C., and Gonzalez S. 1999. Gelation of whey protein concentrate-Cassava starch in acidic conditions. J. Agric. Food Chem. 47: 918-923.

Ma, C.Y. 1983a. Chemical characterization and functionality assessment of protein concentrates from oats. Cereal Chem. 60(1): 36-42.

Ma, C.Y. 1983b. Preparation, composition and functional properties of oat protein isolate. Can. Inst. Food Sci. Technol. J. 16(3): 201-205.

Ma, C.Y., and Harwalkar, V.R. 1984. Chemical characterization and functionality assessment of oat protein fractions. J. Agric. Food Chem. 32: 144-149.

Morrison, R.T., and Boyd, R.N. 1987. Fats. Ch. 37 in *Organic Chemistry: 5th Edition*, R.T. Morrisson and R.N. Boyd (Ed.), p. 1263-1274. Allyn and Bacon Inc., Toronto, Ontario.

Phillips, D.R., and Sternberg, M. 1979. Corn protein concentrate: Functional and nutritional properties. J. Food Sci. 44: 1152-1155.

Phillips, L.G., Whitehead, D.M., and Kinsella, J. (Ed.). 1994. *Structure-Function Properties of Food Proteins*. Academic Press, Inc., Toronto, Ontario.

Pomeranz, Y. (Ed.). 1991. *Functional Properties of Food Components*, 2nd ed. Academic Press, Inc., Toronto, Ontario.

Rhee, K.C., Carter C.M., and Mattil, K.F. 1973. Effect of processing pH on the properties of peanut protein isolates and oil. Cereal Chem. 50: 395-403.

SAS Institute Inc. 1999. SAS/STAT *User's guide*, version 8, SAS Institute, Cary, North Carolina.

Shewry, P.R., Halford, N.G., and Tatham, S. 1994. Analysis of wheat proteins that determine breadmaking quality. Food. Sci Technol. Today. 8(1): 31-36.

Temelli, F. 1997. Extraction and functional properties of barley β-glucan as affected by temperature and pH. J. Food Sci. 62(6): 1194-1197, 1201.

Vani, B., and Zayas, J.F. 1995. Wheat germ protein flour solubility and water retention. J. Food Sci. 60(4): 845-848.

Voutsinas, L.P., Cheung, E., and Nakai, S. 1983. Relationships of hydrophobicity to emulsifying properties of heat denatured proteins. J. Food Sci. 48: 26-32.

Wu, Y.V., Cluskey, J.E., Wall, J.S., and Inglett, G.E. 1973. Oat protein concentrates from a wet-milling process: Composition and properties. Cereal Chem. 50: 481-488.

Wu. Y.V. and Sexon, K.R. 1976. Protein concentrate from normal and high-lysine corns by alkaline extraction: Preparation. J. Food Sci. 41: 509-511.

Wu, Y.V., Sexon, K.R., and Wall, J.S. 1976. Triticale protein concentrate: Preparation, composition and properties. J. Agric. Food Chem. 24(3): 511-517.

Wu, Y.V., Sexon, K.R., and Sanderson, J.E. 1979. Barley protein concentrate from high-protein, high-lysine varieties. J. Food. Sci. 44: 1580-1583.

Yasumatsu, K., Sawada, K., and Moritaka,S. 1972. Whipping and emulsifying properties of soybean products. Agr. Biol. Chem. 36(3): 719-727.

6. CONCLUSIONS AND RECOMMENDATIONS

In North America, barley as a human food source is limited to pot barley in soups and stews. Its major use is in the animal feed and brewing industry. Barley is unique because its major components such as β-glucan, lipids, proteins and starch may positively affect the nutritional, functional, structural and health aspects of a given food system. Over the past decade, most of the research on barley has focused on the health benefits of β-glucan and structural aspects of starch. Barley proteins, which account for 8-20% (w/w) of the barley grain, have received minimal attention due to the abundance of animal, milk, egg and now soy proteins.

From the point of view of the economic feasibility of a barley fractionation process, optimizing the extraction conditions of barley components is necessary to ensure that the entire grain is utilized to its fullest extent with minimum waste streams. Pearling (dry method of separation) of barley can be utilized to separate the grain into bran and endosperm fractions with each having its own unique chemical make-up. In this thesis research, pearling level of 19-21% for both Candle (waxy) and Condor (regular) barley varieties was found to be optimum in concentrating protein and lipids in the 19% pearling flour (19% PF) and enriching β-glucan and starch in the 19% pearled grain flour (19% PGF). The distribution of protein classes (albumins, globulins, hordeins and glutelins) within a barley grain is dependent on both variety and the extent of pearling, which affects the amino acid profile in various grain tissues. For both Candle and Condor barley, the globulin content was higher in 19% PF as compared to the whole grain flour (WGF), whereas the hordein content was higher in the 19% PGF. Pearling did not affect the concentrations of albumins and glutelins as they were similar in WGF and both pearling fractions. In Candle barley, 50-51% of albumins and globulins were present in 19% PGF, but in Condor, 60% of albumins were located in 19% PGF while 64% of globulins were present in the 19% PF. The findings indicate that water- and salt-soluble proteins (albumins and globulins) were present in various regions of the grain including the endosperm and aleurone (bran) tissues, whereas storage proteins (hordeins and glutelins) were mostly concentrated (>70%) in the endosperm.

The 19% PGF in both Candle and Condor barley was high in glutamic acid and low in lysine content, which is characteristic of the hordein proteins. In 19% PF of both barley varieties, the concentration of essential amino acids (EAA's) were either slightly decreased or higher as compared to WGF. Overall, in Condor, the EAA profile of albumins, globulins, hordeins and glutelins was higher in 19% PF as compared to the respective four protein classes obtained from WGF and 19% PGF. However, in 19% PF of Candle, the four protein fractions had lower EAA profile as compared to WGF and 19% PGF. Also for Candle, the distribution of lysine was higher in the globulin protein fraction of WGF and 19% PGF as compared to the lysine content in the four protein classes of 19% PF.

Solvent type and pH affect the protein concentrate extracted from grains in terms of protein recovery and protein content. Among the conditions investigated in this research, extraction conditions of 0.035*M* NaOH at pH 11 and 23°C resulted in the best protein recovery and purity for both barley varieties (Candle and Condor) and for the three flour fractions (WGF, 19% PF and 19% PGF). Protein content of the concentrates obtained from 19% PF was consistently lower than that of 19% PGF and WGF for all extraction conditions examined. Increasing the extraction temperature of Candle and

Condor barley 19% PGF to 40°C did not affect protein recovery, but at 50°C, both protein content (75-86%) and recovery (61-69%) were lower as compared to extractions carried out at 23°C where the protein content of the extracts ranged from 79-92% and the recovery of proteins was between 63-74%. The higher concentrations of water- and salt-soluble proteins in the 19% PGF fractions of Candle and Condor barley may in part be due to the presence of the germ, which is very high in albumins and globulins, that may not have been completely abraded into the 19% PF during the pearling step. The differences in the protein content of extracts and recovery within both barley types and flour fractions are due to the differences in protein type (albumins, globulins, prolamins and glutelins) and the quantities present in the grain, which affect protein solubility.

β-Glucan recovery was affected by variety, temperature and pH but was independent of solvent type. For Candle, β-glucan content of the isolated β-glucan fraction and recovery using either NaOH or Na_2CO_3 at pH 11 and 50°C was 68-72% and 66-68%, respectively. For Condor, β-glucan content was between 67-70% with 53-61% recovery. Starch content of the starch fraction and recovery were dependent on the extraction efficiency and solubility of the other major components (i.e. protein, β-glucan and lipids) present within a given flour sample. Starch content of the starch-enriched fraction obtained from Candle and Condor barley using NaOH pH 11/50°C or Na_2CO_3 pH 11/40-50°C was 80-86% while recovery was between 93-95%. Therefore, using either 0.035*M* NaOH or Na_2CO_3 at pH 11 and 50°C will optimize the recovery of proteins, β-glucan and starch in 19% PGF. The effect of temperature on the extraction of barley components from WGF and 19% PF needs to be explored further.

The nutritional quality (based upon EAA content) of WGF, 19% PF and 19% PGF of Candle and Condor barley was better than their corresponding protein concentrates. Alkaline extraction may have caused the deamination of lysine and other amino acids including aspartic acid and cysteine. In this research, the level of EAA's in the protein concentrates extracted from Condor WGF, 19% PF and 19% PGF (except lysine) either met or exceeded the requirements set out by the Food and Agricultural Organization (FAO) and in general, the EAA profile of Condor barley protein concentrates was more favorable as compared to that of Candle barley.

Alkali-extracted protein concentrates from Candle and Condor barley WGF, 19% PF and 19% PGF had high protein (70-92%) and fat (5-15%) (except for Condor WGFpc and 19% PGFpc) contents and were low in β-glucan (0.2-1.5%) and starch (0.2-1.4%). Increasing the protein extraction temperature of 19% PGF to 40-50°C, decreased protein purity by 5-7% but increased β-glucan (0.8-2.0%) content in Candle and Condor barley and starch (2.4-2.6) content only in Candle protein concentrates.

Both Candle and Condor barley protein concentrates displayed a high tendency to bind water (4.2-7.6 g water/g protein) but had somewhat low protein solubility (12-21%) at neutral pH. However, protein solubility for 19% PGF increased to 26-43% with an increase in protein extraction temperature (40-50°C). Protein concentrates from Candle and Condor barley have the ability to bind fat and form stable emulsions (90-100% stable at 23°C and 78-100% stable at 80°C) in oil-water systems. Foaming capacity for 19% PFpc was low (35-47%) for both barley varieties. For Candle barley, 19% PGFpc/40°C had foaming capacity of 430% with 73% stability after 2 hr, whereas for Condor barley, foaming capacity for 19% PGFpc/50°C reached a value of 438% with 88% foam stability after 2 hr.

Differential scanning calorimetry (DSC) analysis confirmed that alkali extraction conditions (0.035*M* NaOH, pH 11 and 23-50°C) affected the molecular conformation of the proteins. Denaturation of proteins should not be considered a negative characteristic, as protein unfolding exposes both hydrophilic and hydrophobic regions on the polypeptide chain, which affects its functionality.

The functional properties of proteins including solubility, swelling, viscosity, water binding capacity, emulsion stability and gelation properties all affect the performance of proteins in various food product applications; such as comminuted meat products like wieners and sandwich meats. For example, Comer and Dempster (1981) used hard wheat flour, soy protein isolate and concentrate and textured soy protein as fillers in comminuted meat products and tested their functionality in various formulations. It is also possible to use barley protein concentrates as binding agents in meat products due to their exceptional ability to absorb water and emulsify fats.

On the other hand, the potential use of barley bran in producing extruded snack products has been evaluated by Dudgeon-Bollinger et al (1997). In their study, snack products (toasted and salted) were successfully extruded from barley WGF and 20% PGF and were well accepted by a trained panel. D'Appolonia and Youngs (1978) examined the quality of bread that used formulations consisting of 10-20% oat bran and oat bran protein concentrate. Sensory panelists preferred oat blended breads over wheat breads. Thus, the potential of using barley bran and pearled grain flour in various food applications is feasible, but very little research has been conducted on the use of barley protein concentrates as nutritional component in foods or as functional ingredients.

The functional properties exhibited by Candle and Condor barley protein concentrates are comparable to, if not better than some of the commercially important cereal and oilseed protein ingredients (corn, soy, wheat, oat and peanut) available in the market place. Alkali-extracted barley proteins show potential for incorporation into a variety of food products such as processed meats, ice cream, cakes and other baked goods. Model systems were used in this research to determine the functional properties of barley proteins; however, additional research is needed to better understand the behavior of barley protein concentrates in multi-component food systems (i.e. interaction with lipids, sugars, complex carbohydrates, vitamins and minerals) to determine if their functional behavior is both consistent and beneficial.

The feasibility of alkali extraction of barley proteins at the pilot plant level needs to be determined, after which, the compositional analysis and functional properties have to be re-evaluated as their properties may differ from those of the protein concentrates extracted at lab-scale processing. Cereal grains, in general, are considered as "nutritional foods" by North Americans. Cereal-based products are low in fat and high in fiber and carbohydrate contents. The U.S. Food and Drug Administration has approved health claims stating that the incorporation of oat β-glucan or soy proteins in the human diet “may reduce the risk of heart disease” since they reduce serum cholesterol levels. Today's consumers are very health conscious and demand food products that will provide them with good nutrition and health benefits. Therefore, besides the consumption of breakfast cereals, there still exists a tremendous market for barley protein concentrates for use in cereal-based nutritional food products that will appeal to the public.

6.1. REFERENCES

Comer, F.W., and Dempster, S. 1981. Functionality of fillers and meat ingredients in comminuted meat products. Can. Inst. Food Sci. Technol. J. 14(4):295-303.

D'Appolonia, B.L., and Youngs, V.L. 1978. Effects of bran and high-protein concentrate from oats on dough properties and bread quality. Cereal Chem. 55(5):736-743.

Dudgeon-Bollinger, A.L., Fastnaught, C.E., and Berglund, P.T. 1997. Extruded snack products from waxy hull-less barley. Cereal Foods World. 42(9):762-766.